MAIN MALARIA VECTOR DISTRIBUTION AND CURRENT STATUS OF INSECTICIDE RESISTANCE IN KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

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LIST OF ABBREVIATION

AChE1- Acetylcholinesterase

ANOVA- Analysis of Variance

ANVR- African Network for Vector Resistance to insecticides

BF- Blood Fed

Bs- Bacillus sphaericus

BSA- Bovine Serum Albumin

Bti- Bacillus thuringiensis israelensis

CDC- Centers for Disease Control

CS- Circumsporozoite

Dbase- Database

DDT- Dichlorodiphenyltrichloroethane

DNA- Deoxyribonucleic Acid

dNTP- Deoxyribonucleotide triphosphate

DoMC- Division of Malaria Control

DVBNTD- Division of Vector Borne and Neglected Tropical Diseases

E- Empty

EDTA- Ethylenediaminetetraacetic acid

ELISA- Enzyme Linked Immunosorbent Assay

EntomoBase- Entomological Database

G- Gravid

GABA- Gamma-Aminobutyric Acid

GPS- Global Positioning System

GST- Glutathion S-Transferases

HBI- Human Blood Index

HBR- Human-Biting Rate

HG- Half Gravid

ICIPE- International Centre of Insect Physiology and Ecology

IRBase- Insecticide Resistance Database

IRS- Indoor Residual Spraying

ITNs- Insecticide Treated Nets

IVM- Intergrated Vector Management

JKUAT- Jomo Kenyatta University of Agriculture and Technology

Kdr- Knock-down resistance

KEMRI- Kenya Medical Research Institute

KU- Kenyatta University

LLINs- Long Lasting Insecticide treated Nets

MFO- Mixed Function Oxidases

Ms- Microsoft

NSE- Non-Specific Esterases

PBS- Phosphate Buffered Saline

PCR- Polymerase Chain Reaction

SDS- Sodium Dodecyl Sulfate

TBE- Tris/Borate/Ethylenediaminetetraacetic acid

UoN- University of Nairobi

WHO- World Health Organization

ABSTRACT

Thorough understanding of malaria vectors distribution is important in generation of spatiotemporal information on species binomics. The current study was carried out with the objective of generating information on the binomics and current insecticide resistance of the competent vectors in Kenya. Additionally, entomological surveillance on the competent vectors was conducted along the Kenyan coast. Data on entomological profiles were obtained from published and unpublished literature searches. Data were abstracted, entered into Microsoft excel and maps were then generated using ArcGIS 10. Regarding entomological surveillance, adult and immature mosquitoes were sampled and then identified to species level. Presence of sporozoites and blood-meal sources were analyzed using CS and blood-meal ELISA respectively. Results obtained from the searches showed that *An. gambiae* s.s. was largely distributed in Western and Coastal region with isolated focal presence in Central Kenya. Similarly, widely distribution was observed for *An. arabiensis* and *An. funestus* while *An. merus* was limited to Kenyan Coast. Insecticide resistance to pyrethroids and DDT was documented in Western Kenya. Results from the entomological surveillance obtained a total of 456 *Anopheles gambiae* s.l. and 148 *An. funestus* s.l. adults and 567 *Anopheles* larvae. *An. arabiensis* was the predominant species in *An. gambiae* complex. A majority of the blood-meal sources were of human origin and sporozoite rates were very low. In conclusion, Entomological database provide valuable data on the species bionomics, and can be used by malaria control managers for routine entomological surveys for policy and strategy. Though resistance to pyrethroids has been detected, pyrethroids remain effective in the control of malaria vectors in Kenya.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Malaria Parasite and Vector

Malaria remains a major public health concern worldwide as it causes a debilitating parasitic infection with profound impact on the socio-economic development of any country or community where the disease occurs (Sachs and Malaney, 2002). The causative organisms of the malaria disease are protozoan parasites belonging to the genus *Plasmodium* of Plasmodiidae family in the Coccidian order (Sinden and Gilles, 2002). The parasites of humans are mainly from two subgenera, *Laverania* and *Plasmodium* with the former consisting of only *Plasmodium falciparum* Welch the most virulent of all human infective parasite while the later is composed of *P. vivax* Grassi and Feletti, *P. ovale* Stephens and *P. malariae* Grassi and Feletti which are less lethal (Sinden and Gilles, 2002). A fifth human malaria parasite *P. knowlensis* Grassi and Feletti has been discovered infecting humans (Cox *et al.*, 2010) but its epidemiology is not yet understood.

Every year, an estimated 1-3 million deaths occurs worldwide as a result of malaria infections with a majority of the mortality cases being recorded in tropical areas of Central America, Asia and Africa particularly in the Sub-Saharan African countries (Rowe *et al.*, 2006) which are hardest hit by malaria infections. According to the Division of Malaria Control (2011), *Plasmodium falciparum* is the predominant species (98.2%)

while *P. malariae* is 1.8 %. However, *P.vivax* may account for up to 40-50 % of infections in the Northern and North Eastern parts of Kenya.

The human malarial parasites are exclusively transmitted by anopheline mosquitoes and the source of infection to the mosquito is almost always from an infected human and then the parasites invade the gut of the vectors to undergo the extrinsic (sporogonic) developmental stage. Other sources by which anopheline mosquitoes become infected are from infected birds and monkeys. Although there about 3,200 mosquito species so far described, some 430 belong to the genus *Anopheles* (Renshaw and Silver, 2001). Only one genus, *Anopheles*, is able to transmit human malaria (Service and Townson, 2002).

In most malarious regions, malaria transmission is mainly dominated and driven by two or three important vector species that are ecologically adapted to reproduce and survive in the area. For example, in the sub-Saharan Africa and Kenya in particular, the most important and predominant malaria vectors include *Anopheles gambiae* Giles complex (especially *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton), *Anopheles funestus* Giles complex and *Anopheles pharoensis* Theobald (Service, 1993). The different *Anopheles* mosquito species show spatial heterogeneity in distribution due to their diverse forms of aquatic breeding habitats although some species are known to predominate and prefer certain habitat types compared to others (Gillies and De Meillon, 1968). Typically *Anopheles gambiae* s.l. breeds more prolifically in temporary and turbid water bodies such as ones formed by rain compared to *An. funestus* which prefer more permanent water bodies (Gillies and De Meillon, 1968). Characteristically, the

breeding habitats are usually shallow unprotected pools that have some degree of oxygen and protected from extreme heat (Gimnig *et al.*, 2001).

1.1.2 Malaria Vector Control

Vector control remains the most preferred strategy for reducing malaria transmission. The two main methods of malarial vector control are Indoor Residual Spraying (IRS) and Insecticide-Treated Nets (ITNs)/ Long Lasting Insecticide treated Nets (LLINs) (Djenontin *et al.*, 2009). Currently, synthetic pyrethroids are the only group of insecticides licensed for use in ITNs (Vezenegho *et al.*, 2009) while pyrethroids and dichlorodiphenyltrichloroethane (DDT) are used in IRS.

The challenges facing the use of insecticides is the development of resistance which has been reported in West and Central African (Santolamazza *et al.*, 2008, Yadouleton *et al.*, 2010) and East African countries including Kenya (Matowo *et al.*, 2010, Mathias *et al.*, 2011). Due to this, other methods are being used as alternatives to insecticides including larval source management, house screening, environmental management and the use of *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) bacteria that disrupts the mid gut lining of mosquito larvae (Charles *et al.*, 1996).

1.1.3 Integrated Vector Management

In an effort to combat the spread and impact of malaria the adoption of a combination of control strategies through Integrated Vector Management (IVM) has gained popularity. An IVM strategy is a rational decision-making process for the optimal use of resources for vector control. It is essentially a management approach to improve the efficiency,

effectiveness and ecologically soundness of vector control interventions given the available tools and resources (Chanda *et al.*, 2008). Advances in geographical information systems (GIS) have contributed to more precise mapping of the distribution of mosquito species, their breeding areas, and disease transmission. These can be used in IVM to guide targeted control efforts, improve cost-effectiveness and minimize unwanted ecosystem disruption or damage.

1.1.4 Application of GIS in Vector- Borne Diseases

GIS are a combination of computer technologies that integrate graphic elements with database information and enable the computation of spatial relationships. GIS makes it possible to collect, manage, analyze and report spatial information about vector borne-diseases. GIS methods has also become integral part in the disease vector surveillance studies especially in identifying environmental factors responsible for pathogen transmission and survival (Moore and Freier, 2005). In studying vector-borne diseases, investigators often use an ecological approach, in which the interaction between human, cultural and natural environments is analyzed to identify factors associated with the survival of infectious agents. Kitron (1998) emphasized the importance of landscape ecology to epidemiologic studies in vector biology diseases and the integration of geospatial tools to provide a flow of spatial data from a variety of sources of analysis purpose.

1.1.5 Mapping the Malaria Vectors

The vast majority of current malaria control efforts use interventions aimed at limiting human-vector contact. In Africa, these interventions are ITNs and IRS. These

interventions are often deployed without a detailed understanding of the bionomics of the local vectors. Thus, appropriate vector control depends on knowing both the distribution and epidemiological significance of *Anopheles* vectors. Vector control managers require fully informed basic knowledge of local anophelines. Attempts have been made to describe and map the *Anopheles* distribution in the Americas (Foley *et al.*, 2008), Europe (Kuhn *et al.*, 2002), Central and South East Asia (Manguin *et al.*, 2008) and in Africa (Coetzee *et al.*, 2000). Recently, these vector distribution maps have been updated (Sinka *et al.*, 2010). The importance of developing vector maps and regular updating the maps is to provide strategic, evidence-based advice for malaria control programmes.

In 2000, African Network for Vector Resistance to insecticides (ANVR) under the auspices of WHO/AFRO was established. ANVR is divided into four sub-networks: West Africa, Central Africa, East Africa and Southern Africa. ANVR was established to monitor, manage vector resistance and establish technical support networks for supporting the implementation of malaria control activities at country level. Distribution maps are then generated that are applied to gauge the importance of emerging insecticide resistance among the dominant vector species.

1.2 LITERATURE REVIEW

1.2.1 Bionomics of the Main Malaria Vectors

Members of the *An. gambiae* complex *and An. funestus* complex are the most important vectors of malaria in Africa. These vectors are *An. gambiae* s.s., *An. arabiensis* and *An. funestus* s.s. (Coetzee *et al.*, 2000, Coetzee *et al.*, 2004). In Africa, the distribution of *An.*

arabiensis is concentrated in the lower rainfall zones, which represent the drier savannah areas (Lindsay *et al.*, 1998). *Anopheles arabiensis* is recorded more often than *An. gambiae* s.s. where rainfall is < 1000mm whereas the reverse is true where rainfall is >1000 mm (Coetzee *et al.*, 2000). *Anopheles arabiensis* occurring in desert areas can be explained by their association with river systems e.g. the Nile in Sudan (Ageep *et al.*, 2009). Where *An. arabiensis* occurs in equatorial rainforest regions, it is usually associated with a history of extensive land clearance e.g. in Benin City, Nigeria (Coetzee *et al.*, 2000).

Anopheles quadriannulatus Theobald species A and B are found in southern Africa and Ethiopia respectively (Habtewold *et al.*, 2008). *Anopheles merus* Donitz occurs along the East African coast while the *An. melas* is distributed along the West African coast (Davidson, 1964). *Anopheles bwambae* occurs in Semliki valley of Uganda (Besansky *et al.*, 2006). *Anopheles funestus* prefers breeding in permanent fresh water and it is distributed in eastern, western, central and southern Africa (Garros *et al.*, 2004, Koekemoer *et al.*, 2006).

In the subsequent sub-topics, the bionomics, ecology and transmission of the main malaria vectors in Kenya will be discussed in details. A summary of the bionomics of the main malaria vectors in Kenya is shown in Appendix 3.

1.2.1.1 *Anopheles gambiae* s.s.

Anopheles gambiae s.s. is mostly distributed in areas of Western province (Wamae *et al.*, 2010) and Nyanza province (Mutuku *et al.*, 2009), closest to Lake Victoria and in the Coast province (Mwangangi *et al.*, 2007) with few presences reported in the more central regions of Kenya (Lehmann *et al.*, 1999).

In western Kenyan highland, *An. gambiae* s.s. is a dominant vector and plays an important role in malaria transmission (Shililu *et al.*, 1998, Ndenga *et al.*, 2006). In a study conducted in the western highland of Kenya, showed stable transmission at the valley bottom and unstable transmission at the hilltop while the mid-hill village was in an intermediate state, it was noted that there was topographic effect on malaria transmission (Githeko *et al.*, 2006). *Anopheles gambiae* s.s. is also a predominant vector at the Kenyan coast (Mbogo *et al.*, 2003). Malaria transmission in coastal Kenya is very heterogeneous and *An. gambiae* s.s. contributes most to the transmission of *P. falciparum* along the coast (Mbogo *et al.*, 2003).

Anopheles gambiae s.s. is among the world's most efficient vectors of human malaria and their unique bionomics, particularly their anthropophilic, endophagic and endophilic characters, guarantee a strong mosquito-host interaction, favorable to malaria transmission (Takken *et al.*, 1999). Along the Kenyan coast, *An. gambiae* s.s. is highly anthropophilic and the human blood index is very high therefore, the vector can be controlled using ITNs (Mbogo *et al.*, 1993). In western Kenyan highland, *An. gambiae* s.s. is highly endophagic and anthropophagic (Githeko *et al.*, 1994) and ITNs can be used

as a protective measure against the vector. The peak biting activity is between 2300-1000 h (Aniedu *et al.*, 1997) and the flight range of *An. gambiae* s.s. is 2 km (Gillies and de Meillon, 1968) whereas the maximum flight is 14 km (Kaufmann and Briegel, 2004).

Anopheles gambiae s.s. larvae are commonly found breeding in temporary, shallow, well lit, small bodies of water, such as puddles in hoof prints, wheel ruts and small ground pools sites (Minakawa *et al.*, 2005) which are only present after rainfall.

1.2.1.2 *Anopheles arabiensis*

Anopheles arabiensis is ubiquitous in its distribution in Kenya (Minakawa *et al.*, 2002, Mbogo *et al.*, 2003, Muturi *et al.*, 2007). *Anopheles arabiensis* is considered a species of dry, savannah environments and sparse woodland (Coetzee *et al.*, 2000). Working in Mwea, Central Kenya, Muturi *et al* (2008) observed higher densities of *An. arabiensis* in the planned irrigated rice villages compared to non-irrigated villages and concluded that *An. arabiensis* plays a significant role in malaria transmission. At the Kenyan coast, *An. arabiensis* is not a dominant vector and doesn't play a major role in malaria transmission (Mbogo *et al.*, 2003).

Anopheles arabiensis is zoophilic, exophagic and exophilic species (Shililu *et al.*, 2004) but studies at the Kenyan coast has shown that it is highly anthropophilic irrespective of the availability of cattle and other domestic animals (Mwangangi *et al.*, 2003). Also working in Mwea, Central Kenya, Muriu *et al* (2008) observed that *An. arabiensis* was zoophilic and exophagic therefore considered a good candidate of zooprophylaxis which

is a potential malaria control strategy in rice growing areas of Africa. In western Kenya, *An. arabiensis* is largely zoophagic but endophilic (Githeko *et al.*, 1994). The peak evening biting times of *An. arabiensis* can begin in the early evening, 1900 h, or early morning, 0300 h (Tirados *et al.*, 2006) in addition the flight range is 2km (Service, 1997).

Anopheles arabiensis larval breeding habitats are similar to those of *An. gambiae* s.s. generally small, temporary, sunlit, clear and shallow fresh water pools (Minakawa *et al.*, 2005), although *An. arabiensis* is able to utilize a greater variety of locations than *An. gambiae* s.s., including slow flowing, partially shade streams (Shililu *et al.*, 2007) and a variety of large and small natural and man-made habitats. It has also been found breeding in irrigated rice field and application of nitrogenous fertilizer increase mosquito larvae (Mwangangi *et al.*, 2010).

1.2.1.3 *Anopheles merus*

Anopheles merus occurs along the Kenyan coast (Mbogo *et al.*, 2003). *Anopheles merus* is considered a coastal saltwater species but can also be found breeding in inland saltwater habitats (Service, 2008). *Anopheles merus* is not a dominant species along the Kenyan coast and contributes very little to malaria transmission (Mbogo *et al.*, 2003).

Anopheles merus is exophagic and exophilic (Mutero *et al.*, 1984) and Gillies and de Meillon (1968) suggested that *An. merus* shows a preference for animal hosts. Studies conducted by Mwangangi *et al* (2003) at the Kenyan coast showed that *An. merus* is highly anthropophilic and the human blood index is very high. This implies that the

management and control of these vector can be easily targeted using ITNs. The peak biting activity of *An. merus* is between 0000h and 0100h (Mutero *et al.*, 1984).

Anopheles merus larvae are commonly found breeding in shallow brackish pools and marsh or swamp areas along the coast (Coluzzi and Sabatini, 1969).

1.2.1.4 *Anopheles funestus*

Anopheles funestus complex is distributed at the Coast (Mwangangi *et al.*, 2007), in Central regions (Muturi *et al.*, 2010) and Lake Victoria in Western (Munga *et al.*, 2009) and Nyanza (Mutuku *et al.*, 2009) Provinces, Kenya. The vectorial system in the western Kenyan highlands is dominated by *An. gambiae*, whereas *An. funestus* plays a minor role in transmission (Ndenga *et al.*, 2006). Clearing swamps and creating drainage channels for agriculture may negatively affect the abundance of *An. funestus* and at the same time favor the abundance of *An. gambiae* s.s. in the western Kenyan highland (Githeko *et al.*, 2006). In studies conducted at the Kenyan coast by Mbogo *et al* (2003) showed that *Anopheles funestus* is also a predominant vector at the Kenyan coast and plays a more important role in malaria transmission in the southern coastal area. Working in Mwea, Central Kenya, Muturi *et al* (2008) observed higher densities of *An. funestus* in the non-irrigated rice villages compared to irrigated villages and concluded that *An. funestus* plays a significant role in malaria transmission.

Anopheles funestus is highly anthropophilic (Antonio-Nkondjio *et al.*, 2006), endophilic (Gillies and de Meillon, 1968) and endophagic (Oyewole *et al.*, 2007). At the Kenyan

coast, *An. funestus* is highly anthropophilic and the human blood index is very high therefore, this implies that the vector may easily be targeted by using the current available tools for reducing human-mosquito contact (Mwangangi *et al.*, 2003). In the western region of Kenya, *An. funestus* is highly endophagic and anthropophagic (Githeko *et al.*, 1994). In a study conducted in Mwea, Central Kenya, Muriu *et al* (2008) observed that *An. funestus* was substantially anthropophilic and endophagic therefore, may not be a good candidate of zooprophylaxis. Peak biting activity is between 0000h and the early hours of morning (Githeko *et al.*, 1996). The flight range of *An. funestus* is 1km (Gillies and de Meillon, 1968).

A typical *An. funestus* larval breeding habitat is a large, permanent or semi-permanent body of fresh water with emergent vegetation, such as swamps, large ponds and lake edges. Larvae have been found in shaded and sunlit environments (Gillies and de Meillon, 1968). In some areas, *An. funestus* larvae are associated with rice cultivation e.g. Kenya (Muriu *et al.*, 2008).

1.2.2 Insecticide Resistance

Resistance is defined by World Health Organization (WHO, 1957) as the development of an ability or strain of some organisms to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species.

Insecticides are either inorganic or organic in origin. Inorganic compounds include those of fluorine, phosphorus, and sulfur while organic compounds are those of synthetic or botanical origin. The synthetic compounds are divided into four classes; organochlorines

(e.g. DDT, dieldrin) organophosphates (e.g. Malathion, fenitrothion), carbamates (e.g. propoxur) and pyrethroids (permethrin, deltamethrin, lambdacyhalothrin).

1.2.2.1 Development of Insecticide Resistance

Development of resistance depends on the interaction of several factors. According to WHO (1980) and Wood and Bishop (1981) the multiple factors that influence the development of resistance to insecticides can be classified into the following categories: Genetic (mutation rate, relative fitness of genotypes), Reproductive (rate of increase, and fluctuations in population size, generations per year), Behavioural/Ecological (migration in and out of exposed population, avoidance of the insecticide) and Operational (persistence of insecticide, dosage of insecticide taken up by exposed insects).

1.2.2.2 Mechanism of Insecticide Resistance

The mechanisms of physiological resistance in insects are; altered target site of action for the insecticide, detoxification of the insecticides, reduced penetration of the insecticides and insecticide avoidance.

There are three major target sites for most insecticides: the λ-aminobutyric acid (GABA) receptor is the target of cyclodiene insecticides, the voltage-dependent sodium channel is the target site for DDT and pyrethroids, and acetylcholinesterase (AChE1,) is quasi-irreversibly inhibited by organophosphorous and carbamate compounds, which are substrate analogues (Djogbe 'nou *et al.*,2008). The insecticides for malaria control target only two neurological sites, the voltage-dependent sodium channel and acetylcholinesterase.

The voltage-dependent sodium channel target site insensitivity results from point mutations in the voltage-gated sodium channel gene which is a target site for DDT and pyrethroids. This mechanism is also termed knock-down resistance (*kdr*) (Kerah-Hinzoumbé *et al.*, 2008). Two known mutations within the sodium channel gene, also known as *kdr* mutations do occur (Vezenegho *et al.*, 2009). *Kdr* mutation is due to a single nucleotide polymorphism in the gene encoding sub-unit II position 1014 of the sodium channel gene and leads to the substitution of leucine for phenylalanine (L 1014 F). On the other hand, *Kdr* mutation in the same amino acid results in leucine-serine substitution (L 1014 S) (Santolamazza *et al.*, 2008).

Acetylcholine is the transmitter at central nervous system synapses in insects. In order for the nervous system to operate properly it is necessary that, once the appropriate message has been passed, excess acetylcholine should be removed from the synapse, both to prevent repetitive firing and to allow a succeeding message to be transmitted. This removal is effected by the enzyme acetylcholinesterase (AChE1), which catalyses hydrolysis of the ester bond. Organophosphate and carbamate insecticides inhibit the esterase. The result of this inhibition is that acetylcholine accumulates in the synapses so that nerve function is impaired. The gene that encodes for AChE1 is *ace 1* (Khajehali *et al.*, 2009). Insecticide resistance mechanism is due to a single amino acid substitution, from a glycine to a serine at the position 119, in the AChE1 catalytic site (G119S) (Djogbénou *et al.*, 2009).

Resistance to cyclodiene insecticide dieldrin has been associated with mutations occurring in the M2 transmembrane domain of the GABA receptor. Two mutations associated to the dieldrin resistance may occur, either mutation conferring the substitution of alanine296 to glycine or mutation of the same codon conferring the substitution of alanine to serine (Brooke *et al.*, 2006).

The second mechanism of insecticide resistance is the detoxification of the insecticides. The major enzymatic families associated with resistance include non-specific esterases (NSE), mixed-function oxidases (MFO)/ monooxygenases and glutathion S-transferases (GST) (Hemingway and Ranson, 2000). The insect may produce increased quantities of these enzymes, which either metabolize the insecticide or sequestrate the molecules so they cannot function (Matowo *et al.*, 2010). Mixed function oxidases (MFOs) are associated with cross-resistance between DDT and Pyrethroids (Fonseca-González *et al.*, 2009). Non-specific esterases (NSEs) are commonly involved in the detoxification of organophosphates and carbamates, high levels of these enzymes have also been associated with resistance to permethrin (Fonseca-González *et al.*, 2009 and Vulule *et al.*, 1999). Metabolism mediated by GST has been implicated in DDT and organophosphate resistance.

Another mechanism of insecticide resistance is reduced penetration of insecticides which can be achieved through cuticular thickening. Thicker cuticles lead to slower rates of insecticide absorption, which is likely to enhance the efficiency of metabolic detoxification. Decreased penetration on insecticides would allow ample time for

detoxifying enzymes to metabolize the chemical and therefore would be less effective (Plapp *et al.*, 1976).

The irritant property of some insecticides can cause a proportion of insects to leave sprayed surfaces before acquiring a lethal dose, so that repeated contact is required before mortality occurs. The evasive habits due to the presence of insecticides are often referred to by the term" behaviorist resistance ",which means development of the ability to avoid a dose which would prove lethal (WHO, 1957). Behaviorist resistance should be reserved for populations that have been changed by selection; it should not be applied to populations which show pronounced irritability or evasive habits as their normal reaction to certain insecticides. In the case, where those habits are "natural", the term "protective avoidance" is used (WHO, 1960).

1.2.2.3 Impact of Insecticide Resistance in Malaria Control

The two main methods of malarial vector control are indoor residual spraying (IRS) and insecticide-treated nets (ITNs)/ long-lasting impregnated nets (LLINs) (Djenontin *et al.*, 2009). Currently, synthetic pyrethroids are the only group of insecticides licensed for use in ITNs (Vezenegho *et al.*, 2009) owing to their strong insecticidal activity at low concentrations and their low mammalian toxicity (Zaim *et al.*, 2000). The six pyrethroid insecticides currently recommended for mosquito-net impregnation are permethrin, deltamethrin, cypermethrin, λ-cyhalothrin, α-cypermethrin and cyfluthrin (WHO, 2005). World Health Organization have approved the following insecticides for IRS; DDT, fenitrothion, bendiocarb, lambdacyhalothrin and permethrin. However, malaria vector control interventions have been hampered by the emergence of insecticide resistance to

chemicals used for both these interventions (Yadouleton *et al.*, 2010). The major impact of insecticide resistance in malaria control is that it prevents the achievement of malaria eradication and threatens long term ability to control malaria vectors (Kelly-Hope *et al.*, 2008).

In South Africa, malaria vector control failure due to metabolic-based resistance on pyrethroid and carbamate insecticide efficacy has been reported (Hargreaves *et al.*, 2000) and also in Mozambique (Brooke *et al.*, 2001). This resistance is closely associated with the presence of a high level of oxidase activity and sometimes conferring cross-resistance to the carbamate insecticide in the local vector *An. funestus* (Brooke *et al.*, 2001, Dabire *et al.*, 2006). South Africa switched from DDT to deltamethrin for indoor residual house spraying in 1996, oblivious that the populations of *An. funestus* in southern Mozambique were resistant to pyrethroids (Hargreaves *et al.*, 2003). This resulted in malaria epidemic in 1999/2000 which was the worst epidemic experienced in South Africa but it was brought under control by reverting to DDT spraying (Hunt *et al.*, 2005). In Equatorial Guinea the use of pyrethroid in IRS failed to control *kdr* resistant *An. gambiae* and thus it was withdrawn from use in 2004 (Sharp *et al.*, 2007).

Studies have shown that a high L1014F *kdr* frequency in *An. gambiae* s.s. populations of the Ivory Coast had no effect on the effectiveness of pyrethroid-treated nets (Asidi *et al.*, 2004) in addition, metabolic resistance in *An. gambiae* s.s. of Cameroon did not influence the personal protection afforded by ITNs sustained by the strong deterrent effect of the insecticide, to which metabolic resistant vectors are still susceptible (Etang

et al., 2007). Thus, the insecticide resistance conferred by the two mechanism, *kdr* and metabolic resistance impact on the reduced efficacy of the insecticide used or may not reduce the effectiveness of the insecticide.

Corbel *et al* (2004) showed that nets treated with high permethrin concentrations provided better blood feeding prevention against pyrethroid-resistant *An. gambiae* than did lower concentrations. Therefore, insecticide resistance leads to an increase of the dose of the insecticide or the frequency of application and this leads to possible environment contamination.

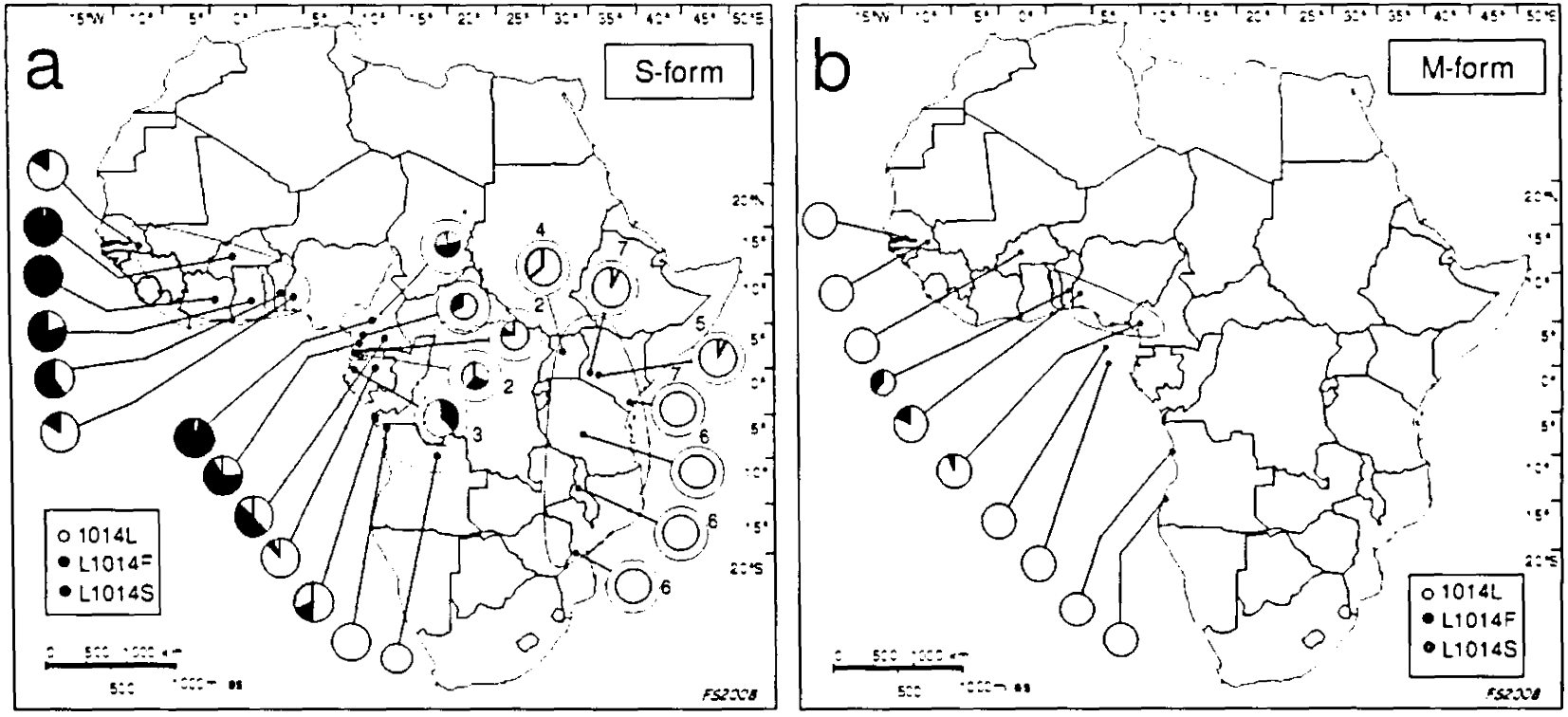
South Africa re-introduced DDT in 2000 which had been replaced by synthetic pyrethroids in 1996. The switch was due to metabolic resistance of *An. funestus* to the synthetic pyrethroid (Craig *et al.*, 2004). Despite the successes achieved, the continued use of DDT in KwaZulu-Natal is being threatened due to the emergence of resistance, and pressure from the communities (Maharaj *et al.*, 2005). Thus, subsequent replacements of insecticides can led to the development of multi-resistant malaria vectors and burden the control programme with increased costs.

The development of new, alternative insecticides is an expensive and long-term endeavour. The current spread of pyrethroid resistance in the major malaria vectors *An. gambiae* and *An. funestus* emphasizes the need to identify alternative insecticides and for the development and implementation of effective and sustainable resistance management strategies. Non-pyrethroid insecticides, such as organophosphates or carbamates, have

potential for use on mosquito nets (Kolaczinski *et al.*, 2000). Unfortunately, a cross resistance to both carbamates and organophospshorous insecticides involving an insensitive acetylcholinesterase has recently been detected in *An. gambiae* from Côte d'Ivoire (N'Guessan *et al.*, 2003) and may, therefore, hamper the use of ITNs in the concerned areas.

1.2.2.4 Insecticide Resistance in Africa

Pyrethroid and DDT resistance is widespread, especially in West Africa (Dabire *et al.*, 2009). The mechanism of insecticide resistance for both insecticides is mainly *kdr* mutation. The L1014F mutation was first described in West Africa (Martinez-Torres *et al.*, 1998). Santolamazza *et al* (2008) analyzed the distribution of the L1014F (West African *kdr* mutation) and L1014S (East African *kdr* mutation) mutations in *An. gambiae* populations from Sub-Saharan Africa west of the Rift Valley (Figure 1.1). The author indicated that, in *An. gambiae* S-form, the L1014F allele is present in the western area of Africa i.e. between Senegal and Nigeria with a *kdr* frequency of greater than 50% in most sites. Also, in West – Central area, Cameroon in the North to Angola in the South, extending eastwards to Uganda both *kdr* mutations are found in the *An. gambiae* S form. The *kdr* frequency is variable from these sites. The *kdr* resistant mutations are likely absent in the Southern countries of Africa. However, Pinto *et al* (2006) observed the East African *kdr* mutation in some parts of Central Africa



Courtesy of Santolamazza et al., 2008

Figure 1.1 Distribution of 1014L, L1014F and L1014S alleles in *Anopheles gambiae* S-form (1a) and M-form (1b) population

In the M- form of *An. gambiae*, the L1014S allele is absent while the S1014F is present in restricted geographic region in the central part of the Gulf of Guinea i.e. Benin, Nigeria and Cameroon (Santolamazza *et al.*, 2008). The first report of the presence of L1014F in M-form was from Benin in 1998 (Akogbeto et al., 1999), where it was shown to have introgressed from sympatric S-form populations (Weill *et al.*, 2000). In Bioko Island (Equatorial Guinea) and Douala (Cameroon) the L1014F mutation may have arisen independently in the M form (Reimer *et al.*, 2005, Etang *et al.*, 2006). Santolamazza *et al* (2008) reported *kdr* frequencies of greater than 75% in Benin and Island of Bioko while less than 20% was observed in Burkina Faso, Nigeria and Cameroon.

The occurrence of L1014F is new and independent in *An. arabiensis* suggesting that its occurrence is not through introgression (Diabate *et al.*, 2004). A study conducted in Lower Moshi, Tanzania detected a low frequency of L1014F in *An. arabiensis* population (Kulkarni *et al.*, 2006) while in Uganda, Verhaeghen *et al* (2006) observed L1014S allele in *An. arabiensis.*

Metabolic resistance has also been observed in some African countries. In north Cameroon, the major malaria vectors, *An. gambiae* and *An. arabiensis*, have developed metabolic resistance to pyrethroids in the absence of known target- site mutations such as *kdr* (Etang *et al.*, 2007). A study conducted in Chad, indicated that *kdr* mutation was not responsible for the resistance in *An. arabiensis* suggesting alternative mechanisms, probably of metabolic origin are involved (Kerah-Hinzoumbé *et al.*, 2008).

In a study in Mozambique, pyrethroid resistance was detected in *An. funestus* populations and linked to elevated levels of monooxygenase activity (Casimiro *et al.*, 2007). Earlier, Casimiro *et al* (2006) working in Mozambique observed that *An. funestus* s.s. is susceptible to DDT and malathion. A high level of pyrethroid resistance was detected in *An. funestus* populations in southern Mozambique and the main mechanism of pyrethroid resistance was because of elevated levels of one or more monooxygenase enzymes (Brooke *et al.*, 2001). It is possible that the GSTs act as a secondary detoxification agent in *An. funestus* populations that show pyrethroid resistance. Elevated GSTs can be a major mechanism of DDT resistance in many *Anopheles*, but no DDT resistance was detected in the *An. funestus* populations with elevated GST activity. It is probable that this AChE mechanism is primarily responsible for the carbamate resistance, with the monooxygenase acting as a secondary mechanism.

A study conducted in southern Mozambique confirmed that, *An. gambiae* s.s. localities fully susceptible to DDT and malathion. A low level of pyrethroid resistance was detected in the populations. Low level resistance to the carbamate, propoxur was also detected in *An. arabiensis* and this was due to increased frequencies of insecticide insensitive acetylcholinesterase, the target site for carbamates and organophosphates (Casimiro *et al.*, 2006)

About a decade ago, Hargreaves and colleagues (2000) recorded resistance to pyrethroids and carbamate insecticides in *An. funestus* from South Africa. Biochemical and synergist assays implicated monooxygenase detoxification as the major resistance mechanism in *An. funestus* samples from South Africa with the possible involvement of elevated GST

activity as a cofactor (Brooke *et al.*, 2001).

Increased levels of non-specific esterases and GST activity found in progeny of the majority of wild-caught *An. arabiensis* females sampled in northern KwaZulu-Natal Province suggest that these enzymes may account for the DDT resistance. Subsequently, Hargreaves *et al* (2003) concluded that due to lack of correlation between mortality data and enzyme level or activity the results were not conclusive.

In surveys conducted by Casimiro *et al* (2007) in Mozambique, low levels of bendiocarb resistance were detected in *An. funestus* populations which was attributed to significantly elevated levels of acetylcholinesterase levels found in the same populations.

In New Halfa, Eastern Sudan, Himeidan *et al* (2008) reported low levels of resistance to DDT, malathion and fenitrothion insecticides in *An. arabiensis.*

In conclusion, there is need to conduct research in these areas to be able to monitor and manage insecticide resistance in these countries.

1.2.2.5 Insecticide Resistance in Kenya

In Kenya, the first reported case of resistance was in the context of insecticide-treated net use in western Kenya where reduced knockdown rates were seen (Vulule *et al.*, 1994). East of the Rift Valley L1014S substitution was first detected in Kenya (Ranson *et al.*, 2000). Stump *et al* (2004) in a study showed that the L1014S allele mutation is found in the *An. gambiae* S form and the *kdr* frequency is about 4% - 8 % in western Kenya but it was not detected in coast province. In addition, L1014S allele was also observed in *An. arabiensis.*

More recently, studies in Central Kenya found no evidence for insecticide resistance in *An. arabiensis* (Kamau *et al.*, 2006).

In an effort to determined baseline information on monooxygenase activity and *kdr* allele frequency in anopheline mosquitoes in the western region, the Great Rift Valley - central region, and the coastal region of Kenya, Chen *et al* (2008) found significant among-population variation in monooxygenase activity in *An. gambiae* and *An. arabiensis* and substantial variability among individuals within populations. Nine of twelve *An. gambiae* populations exhibited significantly higher average monooxygenase activity than the susceptible Kisumu reference strain. The *kdr* alleles (L1014S) were detected in three *An. gambiae* populations, and one *An. arabiensis* population in western Kenya but not in the Rift Valley-central region and the coastal Kenya region. The conservative estimation of *kdr* allele frequency was below 1% in these four populations.

The use of permethrin-impregnated nets for malaria control in Kisumu, western Kenya led to a 2.5-fold increase in the permethrin tolerance of a population of the malaria vector *Anopheles gambiae* s.s. (Vulule *et al.*, 1994). There was no evidence that permethrin tolerance spread to other *An. gambiae* populations or that it reduced the efficacy of permethrin-impregnated nets as a malaria control measure. Vulule *et al* (1999) speculated that use of impregnated nets selected for higher oxidase and esterase levels in *An. gambiae* to metabolize permethrin acquired from the nets. Both oxidase and esterase mechanisms could confer cross-resistance to other pyrethroids.

In a study conducted in Kilifi, Coast Province, Kenya, bioassay, susceptibility and high-performance liquid chromatography results all indicated that the permethrin content applied to the nets was sufficient to maintain high mortality of susceptible vectors, *An. gambiae* s.l. and *An. funestus* (Mbogo *et al.*, 1996).

The status of resistance to DDT, fenitrothion, bendiocarb, lambdacyhalothrin and permethrin was investigated in *An. gambiae* and *An. funestus* mosquitoes from Ahero and Rota in western Kenya and no evidence for resistance was found (Kamau *et al.*, 2008).

In Asembo, western Kenya, permethrin resistance was not detected in *An. gambiae* s.l. and *An. funestus* (Gimnig *et al.*, 2003).

In a recent study conducted by Mathias *et al* (2011) in Seme and Asembo in western Kenya, has shown a sharp increase in homozygote frequencies from complete absence in both locations initially to 80.5% for Seme in 2008 and 91.7% for Asembo in 2010. Also, in his study, *An. gambiae* s.s. were genotyped for the *kdr* L1014S allele from Busia, Malaba, Bungoma, Kakamega, and Kisian and the frequency was high at all sites i.e 79% or greater while a single *An. arabiensis* from Busia that was homozygous for the *kdr* 1014S allele was detected. Data from western Kenya suggest that the rise of the *kdr* allele has had limited impact on the effectiveness of ITNs at least at sites along the lakeshore but monitoring is vital so that strategies can be implemented if and when pyrethroid resistance compromises the effectiveness of ITNs (Mathias *et al.*, 2011).

1.2.3 Malaria Vector Database and Mapping

A range of maps of the dominant *Anopheles* vectors of human malaria will provide a strategic, evidence-based advice for malaria control programmes (Hay *et al.*, 2010). Appropriate vector control depends on knowing both the distribution and epidemiological significance of *Anopheles* vectors (Zahar, 1984). Geographic distribution of the *Anopheles* vector is the result of a complex interaction of biogeography, including biotic (E.g. competition and dispersal) and abiotic factors (e.g. climate and topography) that can vary in both time and space.

To generate the maps, an exhaustive and systematic search of formal and informal literature will be conducted. Then data regarding the main malaria vectors will be extracted and entered into a database. Point maps showing the distribution of the main malaria vectors and insecticide resistance will be generated using ARCGIS. The distribution maps will be improved by combining them with the bionomics of the main malaria vectors. Anopheline vector bionomics is critical in defining the appropriate (and inappropriate) modes of control at the national and local level (WHO, 2004). Information on characteristics of specific larval habitats and range will also be informative. These databases and maps will be used in malaria control and will also help in identifying areas where information is lacking.

1.3 Justification of the Study

The generation of country base maps on the distribution of the main malaria vector species will allow visualization of the distribution data on maps. Base maps on transmission levels and malaria vector densities will enable visualize areas with high,

medium or low transmission levels and vector densities. Mapping of the distribution, transmission levels and densities of malaria vector species will allow for strategic planning of malaria control interventions by the government and non-governmental organization. Knowledge of the distribution, transmission levels and densities of the main malaria vectors will reveal and help define areas that have potential for malaria transmission as well as areas that have no malaria but have the vectors. This will enable control managers to target vector species present at the right time and place appropriately.

Country base maps showing the distribution and status of insecticide resistance of the main malaria vector to insecticides will enable in the management of the resistance and in proper utilization of insecticides.

1.4 Objectives

1.4.1 General Objective

The general aim of this study was to determine the entomological profiles and distribution of the main malaria vector species in Kenya.

1.4.2 Specific Objectives

1. To develop an up to date country database on the distribution, transmission levels and densities of the main malaria vector species in Kenya from peer-reviewed literature sources, unpublished works, reports, and thesis.
2. To develop an up to date country database on the status on malaria vector resistance to insecticides.

3. To conduct entomological surveillance of main malaria vector species in areas where little or no information is available along the Kenyan coast- Kwale, Taveta and Tana River districts.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Entomological Profile and Insecticide Resistance

An exhaustive and systematic desktop search and documentation of formal and informal literature was conducted to build a database on the entomological profiles of the main malaria vectors in Kenya. Only information collected after 1970 to 2010 was evaluated and analyzed. This criterion was used to ensure that the data collected include modern taxonomic species concepts i.e. cytological and molecular techniques which are reliable in identifying sibling species within the *An. gambiae* and *An. funestus* complexes. The development and maintenance of national entomological database is necessary to ensure the systematic and exhaustive collection, interpretation and use of available entomological information.

We have also developed an insecticide resistance database or *IRBase* that consist necessary information regarding the insecticide resistance status of the main malaria vectors to pyrethroids and DDT in Kenya. The country insecticide resistance status produced from these databases will be used by the national malaria control program to monitor and manage insecticide resistance.

2.1.1 Data Type and Source

The entomological profile and database of the distribution of the four main malaria vectors namely; *Anopheles gambiae* s.s., *An. arabiensis*, *An. merus* and *An. funestus* was assembled. According to authoritative reviews (Hay *et al.*, 2010), they were considered to

be among the most important vectors of human malaria by virtue of their competence in preference to feeding on humans, sporozoite rate, abundance and their mean adult longevity (only old individuals incubate the parasite long enough to transmit the disease).

The insecticide resistance profile was also carried out for the main malaria vectors. The insecticide resistance profiles of the main malaria vector species included in the insecticide resistance database were; locality, bioassay method used to test susceptibility, insecticides tested i.e. pyrethroids (permethrin, lambdacyhalothrin, deltamethrin) and DDT.

Published literature in peer reviewed journals was obtained through Pubmed and Hinari searches. Unpublished data, reports and thesis was obtained through linking with the specific authorities such as Division of Malaria Control (DoMC), Division of Vector Borne and Neglected Tropical Diseases (DVBNTD), University libraries i.e. University of Nairobi (UoN), Kenyatta University (KU), Jomo Kenyatta University of Agriculture and Technology (JKUAT) and Research Institutes i.e. Kenya Medical Research Institute (KEMRI) and International Centre of Insect Physiology and Ecology (ICIPE) libraries.

2.1.2 Data Management

The process of the survey included record collection and visit from the University libraries and Research institutes libraries. The survey made use of Pubmed and Hinari searches, reports and thesis.

2.1.2.1 Search Strategy

Malaria entomological and insecticide resistance information available in Kenya were compiled through PUBMED and Hinari searches of literature published in peer reviewed journals, thesis, conference abstract, Ministry of Health reports, and research institutions reports including unpublished sources. The search terms that were used to identify studies that sampled anophelines were *Anopheles* and Kenya while studies that tested for insecticide susceptibility were *Anopheles,* insecticide bioassay, resistance, susceptible, susceptibility test and Kenya.

For each source of data on entomological profile and insecticide status, the author, year of publication, report type, survey location, geo-position were recorded in excel databases and data relating to the vector surveys were extracted, this included date of sampling, duration of sampling in months and/or years, species abundance, species composition, adult or/and larvae collections, identification methods (morphological, PCR, DNA probes, cytological identification) and entomological inoculation rates (EIR) to determine transmission levels. The data relating to insecticide resistance were extracted to include date of sampling, duration of sampling in months and/or years, species composition, adult or/and larvae collections, identification methods (morphological, PCR, DNA probes, cytological identification) bioassay method used to test susceptibility, insecticides tested i.e. pyrethroids (permethrin, lambdacyhalothrin, deltamethrin) and DDT.

The surveyed locations were geo-positioned using the Global Positioning Satellite (GPS) source. Survey sites, whose geographical co-ordinates were not provided by the research

articles, were geo-referenced using digital geographic databases such as Microsoft Encarta, Google Earth, Geonames, and Webdb. Other sources included the national databases e.g. Kenya Enumeration Area centroids database and Topographical Maps. Digital geographic databases are a collection of spatial data and related descriptive data organized for efficient storage and retrieval by many users. The survey sites were keyed in and the co-ordinates of the sites retrieved from databases. Survey sites whose co-ordinates were not retrieved from the digital geographic databases were not included in the entomological and insecticide resistance databases.

2.1.2.2 Data Displays

Data from survey sites entered into the entomological and insecticide resistance databases were imported and converted to database (dbase) and displayed in ArcGIS 10. Digital boundary files were then created for the first level administrative units (Province) to display first, the distribution of the main malaria vectors from each study site. Secondly, the abundance/density/proportions of each species, thirdly, transmission levels determined from EIR, and lastly the status (susceptible, resistance suspected or resistance) of insecticide resistance of each malaria vector against each insecticide in Kenya.

2.1.2.3 Data Analysis

Data analyses were done using Microsoft (Ms) Excel. The proportion of records obtained from provinces, the type of reports, species distribution, collection methods, transmission levels, species abundance and the various identification methods used was determined.

2.2 Entomological Surveillance

It was noted that there were some gaps that existed on entomological profiles in Kenya after collating data in objective 1 therefore; entomological surveillance was conducted in some of the districts to fill the existing gaps.

2.2.1 Study Area and Site

Based on the information collated in objective 1, Kwale, Taveta and Tana River districts in coast province were selected for entomological data collection as shown in figure 2.1. The districts were chosen because they had very little or lacked information on the distribution of the main malaria vectors along the coast. The entomological survey conducted was to fill this gap that existed. The named districts were not the only districts that lacked information in Kenya but were the only ones selected due to limited resources.

Taveta district is one of the new districts in Coast province, Kenya. It was part of Taita Taveta district before it was split into Taita and Taveta districts in 1997. The district lies between latitude 3°07'31.11" S and longitude 37°41'50.70" E (Figure 2.2) and occupies an area of 3,953 km^2. Taveta town is about 109 km West from Voi town off the Nairobi Mombasa road and borders Tanzania to the West, Taveta district to the East, County Council of Olekejuado to the North and County Council of Kwale to the South.

Taveta is mainly inhabited by the Taveta ethnic group. The population of Taveta district is 67,665 according to the 2009 census. The occupation of the people in this district is casual waged labour, mixed farming, livestock and trade/business. The physiographic of the area is a fairly plain terrain that generally slopes towards the south.

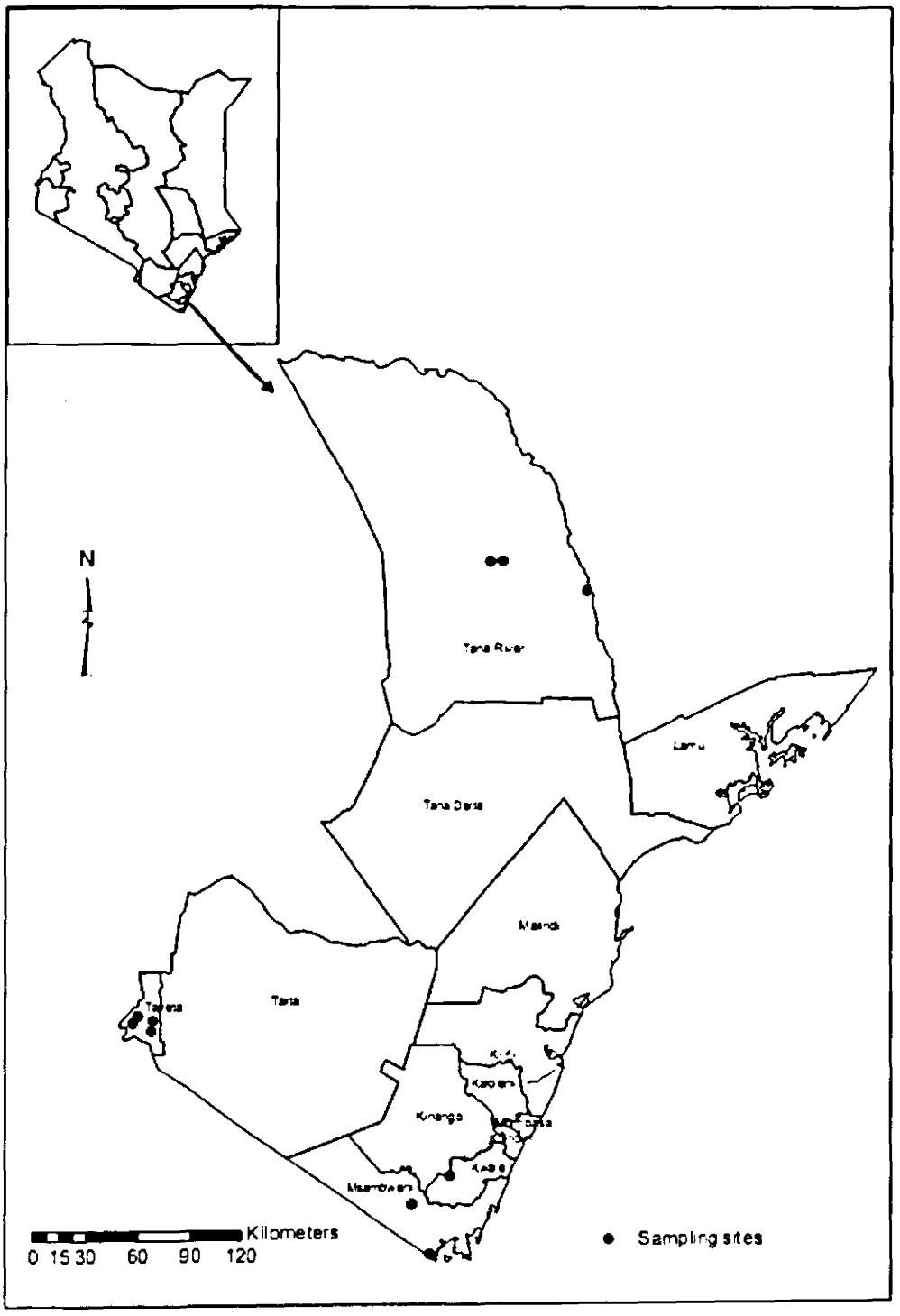


Figure 2.1 Map of Coast province, Kenya showing Taveta, Tana River and Kwale districts where mosquitoes were sampled

The soils in the district are predominantly black cotton and clay which exhibits the characteristic of hardening and cracking during the dry seasons and water logging during the rainy season. The area is about 752.2 m above sea level. Rainfall in the district is inadequate, bimodal and very erratic. The mean annual rainfall ranges between 200 mm and 1,200mm. The long rains fall between March and May while the short rains occur between November and December. Temperature ranges from 21.2°c to 31.0°c and the average relative humidity is 5%. The highest evaporation rate is experienced during the months of January to March.

The agricultural activities in this area include horticulture (growing of tomatoes {*Solanum lycopersicum*}, kales {*Brassica oleracea*} , bananas {*Musa spp.*}), livestock farming (cattle , goat, sheep, poultry and bee keeping), few cash crops which include irrigated rice (*Oryza sativa*) and subsistence farming (growing of maize {*Zea mays*}, beans{ *Phaseolus vulgaris* }, french beans {*Phaseolus vulgaris*} and sugar cane {*Saccharum*}) mainly using irrigated water from the major rivers (River Tsavo, Lumi, Njoro and Kitobo) through construction of canals (Njoro Kubwa and Grogan canal) and spring water that emanates from the foot of Mt. Kilimanjaro. These agricultural activities could influence the breeding sites and the distribution of the malaria vectors. The livestock act as alternative source of blood for the malaria vectors.

The house type of the rural population is mainly stick and mud built houses with either grass thatch or iron sheet roofs (Figure 2.3). The eaves are mostly open leaving ample space for mosquito entry.

Mosquitoes were sampled in four villages; Kiwalwa, Mwarusa, Kimorigo and Njoro in Taveta district as shown in figure 2.2. The rationale that was used to select these villages was nearness to a water body e.g. stream pools, dug ponds, swamps, rivers etc. which are suitable breeding places for malaria vectors and accessibility.

Tana River district is in Coast province, Kenya. The district lies between latitude 1°30'00" S and longitude 40°00'00'' E (Figure 2.2) and occupies an area of 38,446 km^2. Tana River district is about 326 km North West from Mombasa city. The district borders Kitui District to the West, Mwingi to the North West, Garissa to the East, Tharaka Nithi and Isiolo to the North, Lamu to the South, Kilifi and the Indian Ocean to the South East. It is inhabited by the Pokomo, Orma and Wadey, ethnic groups. The Pokomo's are Bantu and predominantly farmers while the Orma's and Wadey's are Cushitic and predominantly nomadic. The population of this district is 143,411 according to the 2009 census.

The physiographic of the area is an undulating plain which is interrupted in a few places by low hills (Minjila, Bilbil, and Madogo). Tana River District generally slopes south-east wards with an altitude that ranges between 0 m along the coastline to 200 meters above sea level on the hills. The soils in the district are clay and alluvial. Tana delta traverses the district and as the river crosses the expansive coastal hinterland, it starts to meander in its lower course forming a large basin. Towards its mouth between Mnazini area and the Indian Ocean, the river creates an extensive delta which is characterized by wetlands. Besides Tana River, there are seasonal rivers in the district. The wetlands and the seasonal rivers could form breeding habitats for the breeding of the malaria vectors.

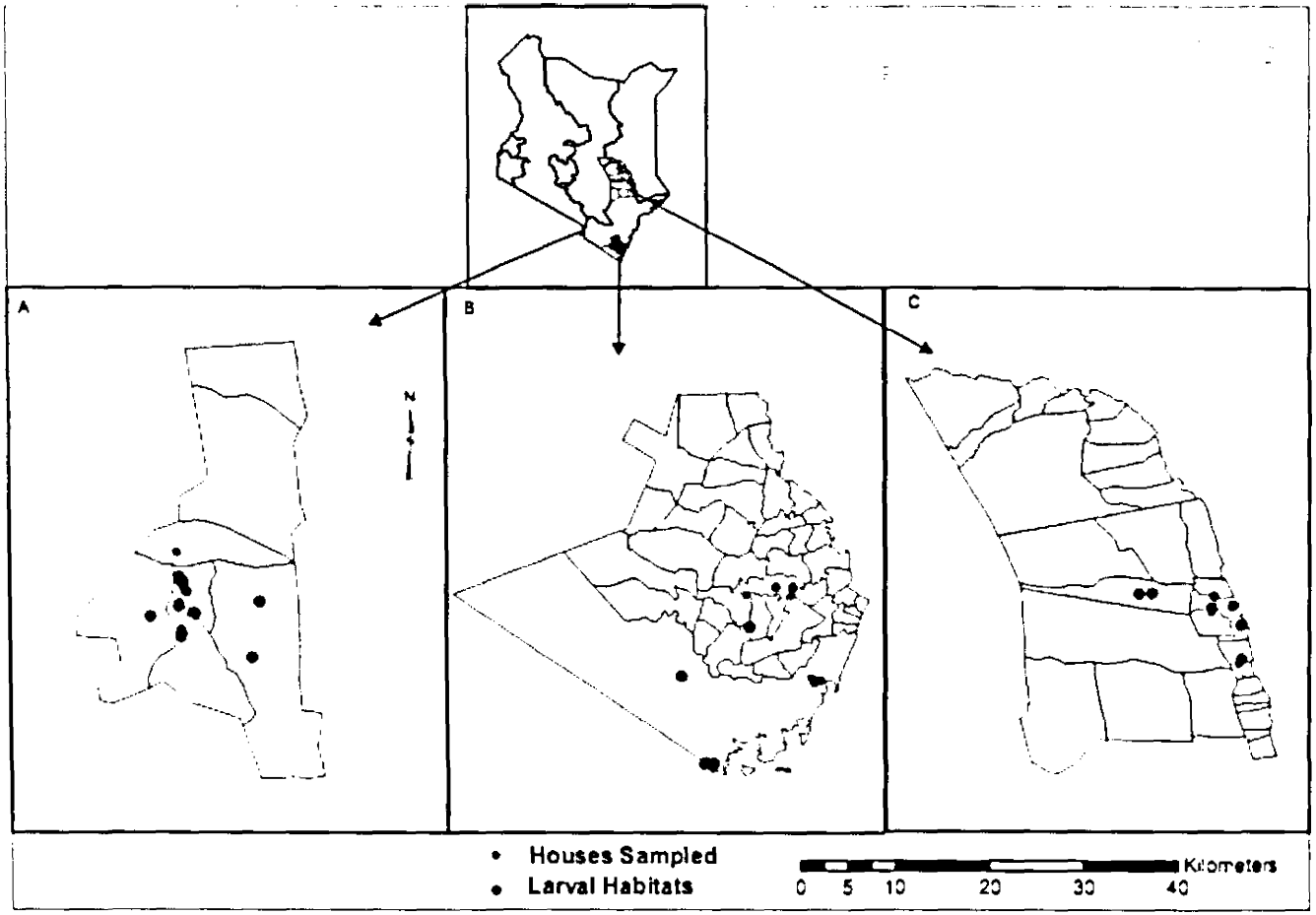


Figure 2.2 Map of Kenya showing Taveta (A), Kwale (B) and Tana River (C) districts study sites that were sampled for mosquitoes.

The district experiences a bi-modal rainfall pattern which is often erratic. Long rains occur in April and May and short rains occur in October and November. The mean annual rainfall range between 220 mm and 500 mm except for the southern part which receive rainfall ranging between 750 mm and 1,250 mm annually. The district is generally hot and dry with temperatures ranging between 21°c and 38°c. The ecological zones of Tana River district are; semi-humid to semi-arid, semi-arid, arid and very arid zones. These zones are important with regard to breeding sites of the malaria vectors.

The agricultural activities in this area use irrigated water mainly from Tana River. Bura and Hola / Tana irrigation schemes have been constructed along the lower Tana basin Delta. The main crop grown in these schemes are maize. Other agricultural activities supported by Tana Delta in the district are growing of rice (*Oryza sativa*), mangoes (*Mangifera*), bananas (*Musa spp.*) and soya beans (*Glycine max*). Fishing, forestry and livestock keeping (cattle, goat, sheep, poultry, donkey, camel and bee keeping) are also supported by the Tana Delta.

The house type of the rural population is mainly stick and mud built houses with either grass thatch or iron sheet roofs (Figure 2.3). There were some house types that are framed wooden poles with grass attached as the wall and roofing material. While other house types are framed wooden sticks with plastic sheet as the wall and the roofing material. The eaves are mostly open leaving ample space for mosquito entry.

Five villages were selected in Tana River District. These include 3 villages (Village 4, 7, 10) in Bura irrigation schemes, Bahati village in Hola irrigation scheme and Chwele

village as shown in figure 2.2. The rationale that was used to select these villages was nearness to a water body and accessibility.

Kwale District is an administrative district in the Coast Province of Kenya; it has an area of 8,293 km^2 with a population of 649,931 persons according to the 2009 census. Kwale is approximately 43.4 km South from Mombasa city. In 2007, Kwale district was split into three districts: Kwale, Msambweni and Kinango districts. The district lies between latitude 04°15'06.02" S and Longitude 39°29'55.97" E (Figure 2.2). The district borders Taita Taveta to the West, Kilifi district to the North West, Mombasa and Indian Ocean to the East and Republic of Tanzania to the South. Kwale is mainly inhabited by Kamba, Duruma and Digo. The main livelihood zones are mixed farming, livestock farming, fisheries and formal employment/tourism.

The district experiences a bimodal rainfall pattern with the short rains occurring between October to December and the long rains occurring between March and June/July. The average annual rainfall ranges between 400mm and 1,200mm. The mean annual temperature is 24.2°c. Kwale district has four major topographical features namely the coastal plain, the Foot Plateau, the Coastal Uplands and The Nyika Plateau. The area is about 400.2 m above sea level.

The soils in this district are predominantly sandy and the agricultural activities include mixed farming, livestock farming (cattle, goat, and poultry) and fisheries.The house type of the rural population is mainly stick and mud built houses with either grass thatch or

iron sheet roofs (Figure 2.3). The eaves are mostly open leaving ample space for mosquito entry.

Three villages were selected for vector sampling in Kwale. These are Jego, Kinango Magaoni as shown in Figure 2.2. The rationale that was used to select these villages was nearness to a water body and accessibility.

Figure 2.3 Stick and mud built house in Taveta, Tana River and Kwale districts

2.2.2 Vector Sampling

A cross-sectional study was conducted in the 3 districts to determine the species composition of the malaria vectors. In Taveta district, 4 villages were sampled for malaria vectors and in each village about 10 houses were randomly selected. In Tana River district, 5 villages were sampled for malaria vectors and in each village about 5-10 houses were randomly selected. In Kwale district, 3 villages were sampled and about 5 houses were randomly selected in each village. In addition to adult sampling, in each village about 3-7 larvae habitats were sampled for malaria vectors. Results from these studies were incorporated in the country database using *EntomoBase*. A schematic diagram representing malaria vector sampling is shown in figure 2.4.

Entomological surveys were conducted in the 3 districts using oral aspiration and Centers for Disease Control (CDC) light traps (WHO, 1975). The immature mosquitoes were collected using the standard dipping technique. The houses and the larval habitats were geo-referenced using a hand held navigational, global positioning system (GPS) receiver (Garmin International Inc.; Olathe KS).

The standard time for day resting indoor collections is 30 minutes per house. Aspiration was done by two collectors from 07:00 h to 10:00 h using an aspirator. Mosquitoes were searched from all accessible places inside the house using torches. The mosquitoes found were orally aspirated with an aspirator placed in paper cups and transported to the laboratory for morphological identification (WHO, 1975).

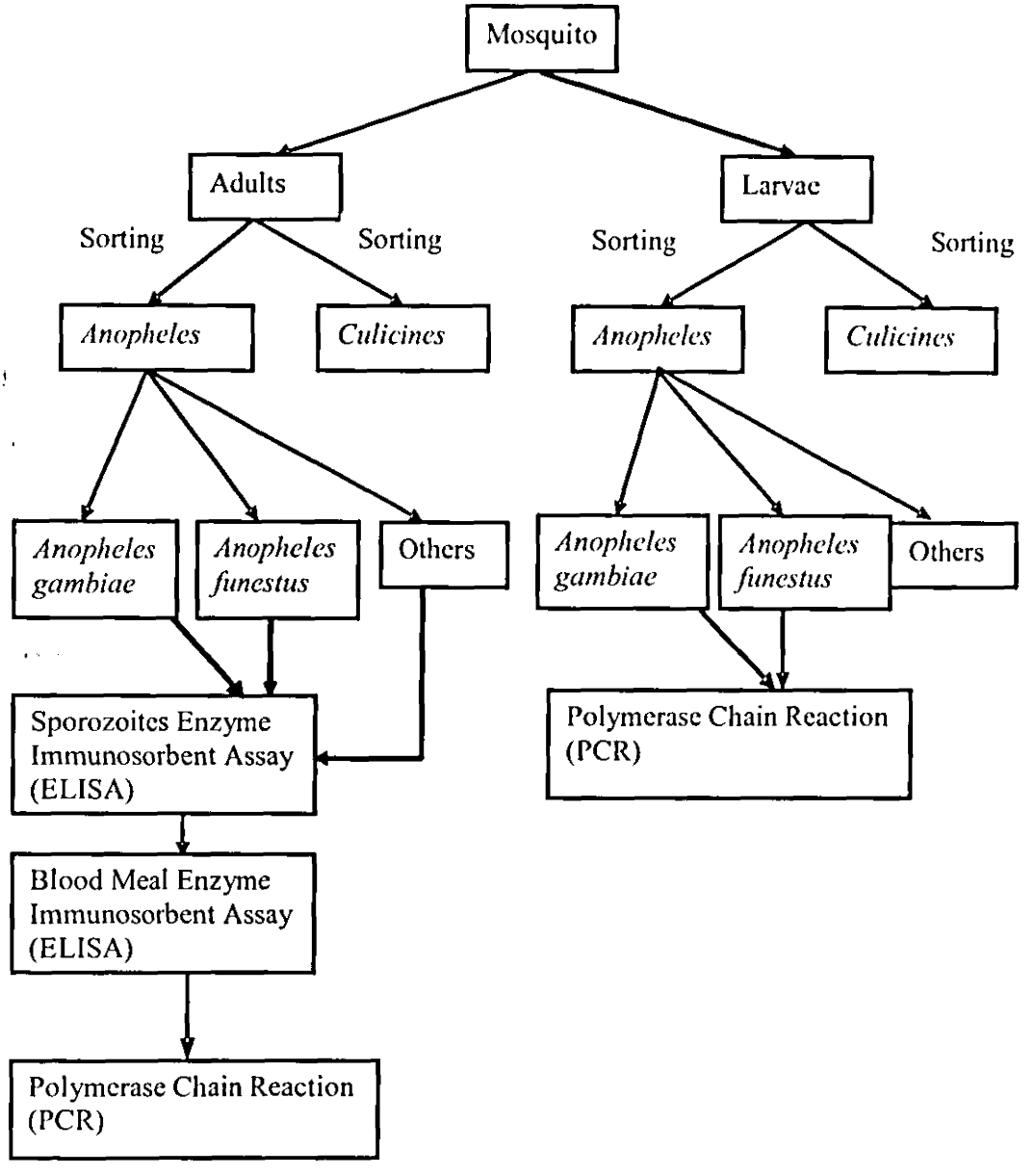


Figure 2.4 Schematic diagram representing malaria vector sampling and laboratory processes

Mosquito collections were sampled using the Centre for Disease Control (CDC) light trap conducted from 18:00 h – 06:00 h inside houses. The light trap was hanged inside houses about 1.5 m from the floor near the bed of the occupants. The mosquitoes collected in the cup holder of the CDC light trap were then transported to the laboratory for morphological identification.

The larval habitat was first inspected for the presence of mosquito larvae. When the mosquito larvae were present 3-30 dips were taken with a standard mosquito dipper (350ml) at each breeding habitat depending on the size. The collections were done using standard dipping techniques. The larvae were kept in sealable polythene bags. Each polythene bag was labeled to show the name of the collection site, date and habitat number. The mosquito larvae collected were transported to the laboratory for morphological identification (WHO, 1975).

2.2.3 Mosquito Identification and Laboratory Processing Procedures

A schematic diagram representing mosquito identification and laboratory processes is shown in figure 2.4.

2.2.3.1 Sorting and Morphological Identification

At the field laboratory, all adult mosquitoes were placed in petri dishes whereas larvae were placed in trays and sorted into *Anopheles* and Culicines. Culicines were discarded because we were only interested in malaria vectors. Morphological characters were used to identify adult *An. gambiae* s.l. and *An. funestus* s.l. and other *Anopheles* mosquitoes

(Gillies and DeMeillon 1968). Collected adult mosquitoes were then placed in tubes, preserved in Carnoy's solution (3:1, acetic acid: Ethanol) and then transported to KEMRI- Kilifi Laboratory for further processing. *Anopheles* larvae were sorted into early instars (L1 and L2), late instars (L3 and L4) and pupae. The late instars were preserved in absolute ethanol and transported to KEMRI- Kilifi laboratory for morphological identification.

In the laboratory, the adult *Anopheles* were sorted physiologically into abdominal status; Empty (E), Blood Fed (BF), Half Gravid (HG) and Gravid (G). All the mosquitoes that were BF and HG were tested for blood meal sources. Individual mosquitoes were cut transversely between the thorax and the abdomen. The anterior portion (head and thorax) were selected for sporozoite ELISA, the BF and HG abdomens were selected for blood meal ELISA and the wings and legs were selected for PCR analysis. In the laboratory, the early larval instars were reared to late instars while the pupae were reared until they emerged into adult for identification. Morphological characters were used to identify *An. gambiae* s.l. and *An. funestus* s.l. and other *Anopheles* larvae mosquitoes (Gillies and DeMeillon 1968). The late larval instars of the complexes were then preserved in absolute ethanol and stored in -20°c until further sibling species identification.

2.2.3.2 Sporozoite ELISA Testing

The anterior portion (head and thorax) of the individual mosquito was placed in 1.5 ml microfuge tube containing 50µl boiled casein blocking buffer (1 litre of blocking buffer contains: 5.0g casein in 100ml 0.1 N sodium hydroxide: 0.1g, thimerosal: 0.01 g phenol

red: 900ml phosphate buffered saline powder (PBS), pH 7.4) with Nonidet P-40 (5µl Nonidet P-40/1 ml blocking buffer). The samples were triturated manually with plastic pestle. 200µl of blocking buffer were then added to the sample to bring the final volume to 250µl per mosquito sample. Samples of the mosquito triturates were then stored at -20^{0}C until further testing. All wells of a 96-well polyvinyl microtitre plate were coated with 0.1 µg *P. falciparum* capture monoclonal antibody diluted in 50 µl PBS/well and incubated for 30 minutes at room temperature in subdued light. The triturates were removed from the freezer and left to thaw at room temperature before they are tested. After 30 minutes, well contents were aspirated and the well filled with 200µl of blocking buffer. After one hour, the blocking buffer were aspirated and 50µl aliquots of each homogenized mosquito triturate added to each well, leaving three wells for negative and three wells for positive controls. After 2 hours of incubation, the mosquito triturates were aspirated and the wells washed two times with PBS- Tween 20 solution and banged to dryness. 50µl of peroxidase conjugated monoclonal antibody were added to each well and incubated for one hour at room temperature. After one hour, the solutions were aspirated and the plates washed three times with PBS-Tween 20 solution and banged to dryness. 100µl peroxidase substrate were then added to each well using an octapete multi-channel pipette and incubated for 30 minutes in subdued light after which samples were assessed visually for positivity.

2.2.3.3 Blood Meal ELISA Testing

The Blood Fed and Half Gravid abdomen of individual mosquitoes were placed in 1.5 ml microfuge tube containing 100µl PBS. The samples were triturated manually with plastic

pestle. 900µl of blocking buffer were then added to sample to bring the final volume to 1 ml per mosquito sample. 50µl mosquito triturates were then added to wells of polyvinylchloride 96- well microtitre plates and incubated overnight at room temperature. Each plate was then washed twice with PBS containing Tween 20 (PBS-TW 20). This was followed by the addition of 50µl host specific conjugate (antihuman igG, H&L) diluted 1:2,000 (or 1:250 for bovine) in 0.5% boiled casein containing 0.025% Tween 20. The boiled casein was prepared by dissolving 5g casein in 100ml 0.1 N sodium hydroxide by boiling, adding 900 ml PBS, adjusting pH to 7.4, adding 0.1g Thimerosal (sodium ethylmercurithiosalicylate) and 0.02 g phenol red, and storing at 4° c. After one hour, wells were washed three times with PBA- Tween 20, and 100 µl of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulfonate]) peroxidase was added to each well. The dark green positive reactions for peroxidase or dark yellow reactions for phoshotases were assessed visually after 30 minutes. A second host source was determined in the same microtitre plate where mosquitoes were screened for human blood. The second conjugate, phosphotase-labelled anti-bovine IgG (1:250 dilution of 0.5 mg/ml stock solution) was added to the peroxidase-labelled antihuman IgG solution. Blood meals were screened first for human IgG by the addition of peroxidase substrate. After 30 minutes, the plates were read, and the wells washed 3 times with PBS-Tween 20, and 100µl phosphatase substrate was added to each well. Plates were read for 1 hour to determine positive bovine reactions. Non-reacting samples were then tested for goat. For each test, 1:500 dilutions of human, cow and goat serum were added to the conjugate solution to reduce backgroung absorbance. Each plate contained control serum samples (1:500 dilution in PBS) of human, cow and goat ground in PBS at the same dilution as test samples.

2.2.3.4 DNA Extraction

DNA was extracted from wings and legs of *An. gambiae* s.l. and *An. funestus* s.l. adults and DNA of the larvae using Collins *et al* (1987) method.

The mosquito legs, wings and larvae were placed into a 1.5 microfuge tube. The portion of mosquito was homogenized with 100µl 0.08 M NaCl, 0.16 M sucrose, 0.06 M Ethylenediaminetetraacetic acid (EDTA), 0.5% Sodium Dodecyl Sulfate (SDS), 0.1 M Tris-Cl, pH 8.6. The tubes were then placed in a water bath at 65°c for 25 minutes. 14µl of 8M potassium acetate was added to a final concentration of 1 M and vortexed to mix. The samples were cooled in ice for 30 minutes then centrifuged at 14,000 rotations per minute (rpm) for 10 minutes. The supernatants were transferred to new clearly labeled microfuge tubes, taking care not to disturb the precipitation layer. 200µl of cold 95% ethanol was added and chilled at –20°c overnight. Spinning was then done in a microfuge at 14,000 rpm for 20 minutes. Thereafter, ethanol was poured off. The pellet was washed with 200µl of 70% ethanol and ethanol was poured off. A similar washing was done using 200µl of 95% ethanol. The tubes were then inverted on absorbent and allowed to air-dry overnight. The pellets were then re-suspended in 10 mM Tris, 1 mM EDTA, pH 8.0.

2.2.3.5 Species Identification using PCR

Identification of sibling species of *Anopheles gambiae* complex and *Anopheles funestus* complex was conducted using the method described by Scott *et al* (1993).

For *Anopheles gambiae* complex, amplification reactions were performed in volumes of 15µl containing 3µl of template DNA. 2.5 µl of 10 x reaction buffer number 1,200 µM of each dNTP,1 mM $MgCl_2$, 0.625 units of AmpliTaq polymerase, 6.25 ng of primer GA, 12.5 ng of primer UN, 18.75 ng of primer AR. *Anopheles gambiae* complex ribosomal DNA (rDNA) intergenic spacer species-diagnostic primers were as follows: GA: 5' CTG GTT TGG TCG GCA CGT TT 3', AR: 5' AAG TGT CCT TCT CCA TCC TA 3', UN: 5' GTG TGC CCC TTC CTC GAT GT 3' (Scott *et al.*,1993). The lengths of the sequences in nucleotides amplified between UN and each of the two species-specific primers is 315 for *An. arabiensis* and 390 for *An. gambiae*. Sufficient sterile water was added to give a total volume of 25 µl. Amplification was performed in a Perkin Elmer 9600 Cetus Thermo cycler programmed as follows: 15 s of denaturing at 94°c, 10 seconds of annealing at 60°c, 20 seconds of extension at 72°c and the cycle was repeated 30 times. There was a final extension at 72°c for 10 minutes.

For *Anopheles funestus* complex, PCR conditions were as follows: 12.5 µl reactions contained the following: 1.25 µl 10 × reaction buffer (500 mMKCl, 100 mMTris-HCl pH 8.3), 1.5mM $MgCl_2$, 200 µM of each dNTP, and 0.5 units thermostable taq DNA polymerase, 3.3 pmol/primer of each primer. *Anopheles funestus* complex ribosomal DNA (rDNA) internal transcribed spacer 2 diagnostic primers were as follows: UV: 5' TGT GAA CTG CAG GAC ACA T 3', FUN: 5' GCA TCG ATG GGT TAA TCA TG 3', VAN: 5' TGT CGA CTT GGT AGC CGA AC 3', RIV: 5' CAA GCC GTT CGA CCC TGA TT 3', PAR: 5' TGC GGT CCC AAG CTA GGT TC 3', LEES: 5' TAC ACG

GGC GCC ATG TAG TT 3' (Koekemoer *et al.*, 2002). The lengths of the sequences in nucleotides amplified were *An. funestus* (≈505bp), *An. vaneedeni* Gillies and Coetzee (≈587bp), *An. rivulorum* Leeson (≈411bp), *An. leesoni* Evans (≈146bp), and *An. parensis* Gillies (≈252bp). Amplification was performed in a Perkin Elmer 9600 Cetus Thermo cycler programmed as follows: 5 minutes of denaturing at 94°c, 30 s of denaturing at 94°c, 30 seconds of annealing at 50°c, 30 seconds of extension at 72°c and the cycle was repeated 30 times. There was a final extension at 72°c for 10 minutes.

2.2.3.6 Agarose Gel Preparation and Electophoresis

After the PCR was complete, the entire reaction was removed, mixed with a standard agarose gel loading buffer containing a small amount of bromophenol blue, and electrophoresed through a 3% agarose-Tris-borate-EDTA gel containing ethidium bromide. The amplified fragments were visualized by illumination with short wave ultraviolet light.

2.2.3.7 Data Management and Analysis

In the field, all field information was recorded in the field forms and the data were entered into Ms Excel files. The forms were checked for accuracy, and then kept in a file in the laboratory. In the laboratory, laboratory processes were recorded in the laboratory processing forms which were also kept in a separate file and the data were then entered into Ms Excel file. The field forms are shown in Appendix 1 and 2.

Statistical analysis was done using Ms Excel. The proportion of the adult mosquitoes, larvae, sporozoite rates and blood meals sources was determined. Household mosquito data within each village were used to calculate the mean household mosquito density and the corresponding variance at that village. *Anopheles* larvae collected from each habitat was used to calculate the larvae density at each village. Analysis of variance (ANOVA) was conducted with log-transformed data to determine whether mean household mosquito densities per village differ among the districts.

CHAPTER THREE

3.0 RESULTS

3.1 Distribution of the Main Malaria Vectors

A total of 780 study sites of *Anopheles* vector species were identified across Kenya for sampling done between 1975 and 2010 as shown in Table 3.1 and Figure 3.1. Of these, 614 (78.72%) of the site-specific data were obtained from peer-reviewed published sources (journal articles) following in reducing order by unpublished work 66 (8.46%), doctoral and masters theses 58 (7.44%), while government reports and conference abstracts provided 38 (4.87%) and 4 (0.51%) respectively. From these scientific data searches, 508 study sites had been surveyed for adult vectors, 178 of which were investigated using only larval sampling from larvae breeding sites, 91 sites for both adult and larval vector sampling and 1 sites were investigated for pupal stages only. From these studies species identification was based on morphological identification at 315 (40.38 %) sites, PCR methods 57 (7.31 %) sites, cytogenic method 2 (0.26%) sites, DNA probes at 6 (0.77%) sites and a combination of methods 397 (50.9 %) sites. Data were extensively collected around malaria research centres in Kilifi, Malindi, Kwale, Suba, Siaya, Bondo, Kisii, Kirinyaga and Kisumu.

Within *An. gambiae* complex, *Anopheles arabiensis* recorded the highest number of occurrences (351 sites) followed by *An. gambiae* s.s. (331 sites) and *An. merus* (73 sites) as shown in figure 3.1.

Table 3.1 Study sites reporting the distribution of main malaria vector species in Kenya

Species	*An. gambiae* s.s.	*An. arabiensis*	*An. merus*	*An. funestus*
Survey Period				
1975 – 2010	331	351	73	476
Mosquito Stages Sampled				
Adults	247	223	62	349
Larvae	56	97	10	63
Adults/Larvae	26	29	1	61
Pupal	1	1	0	1
Province				
Central	3	70	0	49
Coast	110	93	73	177
Eastern	4	7	0	4
Nairobi	1	11	0	1
North Eastern	0	0	0	0
Nyanza	163	132	0	204
Rift Valley	14	22	0	15
Western	36	16	0	26

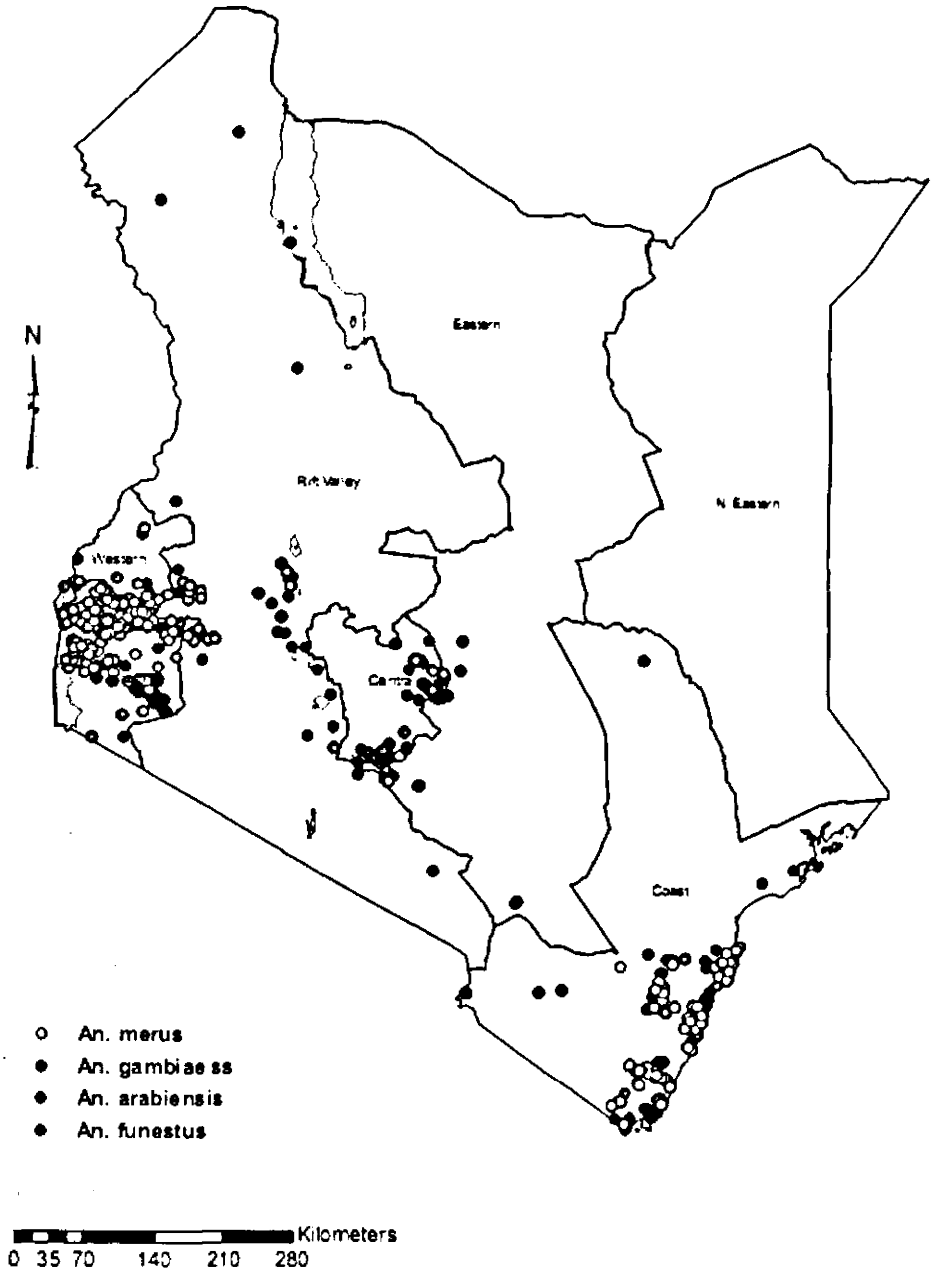


Figure 3.1 Distribution of the main malaria vectors in Kenya

However, a substantial number of sites recorded presence of *An. gambiae* s.l. (279 sites) without clear distinction of the specific species within the complex and this were largely during the earlier years (1971) when tools of distinguishing the species were not well developed. *Anopheles funestus* (Figure 3.1) was recorded in a total of 476 sites distributed in specific sites along the coastal, central and western regions of the country. Other species that were recorded were *An. pharoensis* (60 sites) and *An. nili* (18 sites).

3.2 Transmission Levels of the Main Malaria Vectors

Anopheles gambiae s.s. showed high transmission levels at the coast and western regions (Figure 3.2) while *Anopheles arabiensis* showed high levels of transmission only in Nyanza province (Figure 3.3). *Anopheles merus* played minor role in malaria transmission at the coast (Figure 3.4) while *An. funestus* showed medium to high levels of transmission at the coast and Nyanza provinces (Figure 3.5).

3.3 Densities/Abundance and Proportions of the Main Malaria Vectors

Anopheles gambiae s.s. densities were high at the coast and some parts in nyanza provinces but generally low in the western region of Kenya (Figure 3.6). High densities of *Anopheles arabiensis* were reported in Mwea, Kirinyaga district in central Kenya and some regions of rift valley while coast and western region reported low densities (Figure 3.7). Very low *An. merus* densities were reported at the Kenyan coast except in Malindi district where where it was common (Figure 3.8). Very high densities of *An. funestus* were recorded in in Kisumu district in Nyanza province and Kwale and Kilifi districts in

coast province (Figure 3.9). The proportion of the main malaria vectors in Kenya is shown in Figure 3.10. *Anopheles gambiae* s.s. is a dorminant vector in Western region and coastal Kenya mostly in Malindi and Kilifi districts. *Anopheles arabiensis* is dominant in; Mwea irrigation scheme in Central Kenya, Tana River district in Bura and Hola irrigation schemes and in Rift Valley province in Kericho district, Nandi district and the drier regions of Baringo and Koibatek districts. The most dorminant vector in Taveta and Kwale districts is *An. funestus*.

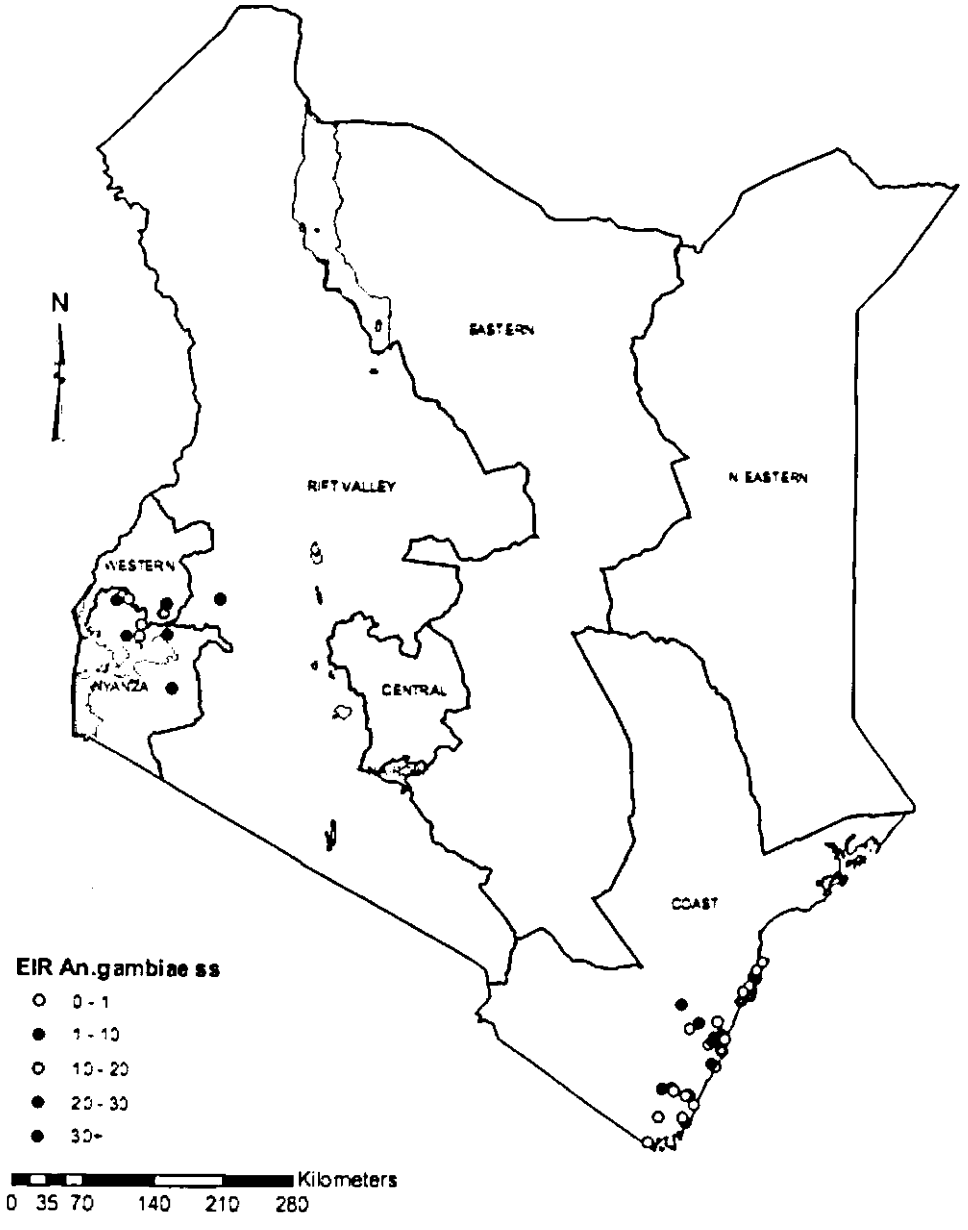


Figure 3.2 Annual Entomological Inoculation Rate for *Anopheles gambiae* s.s. in Kenya

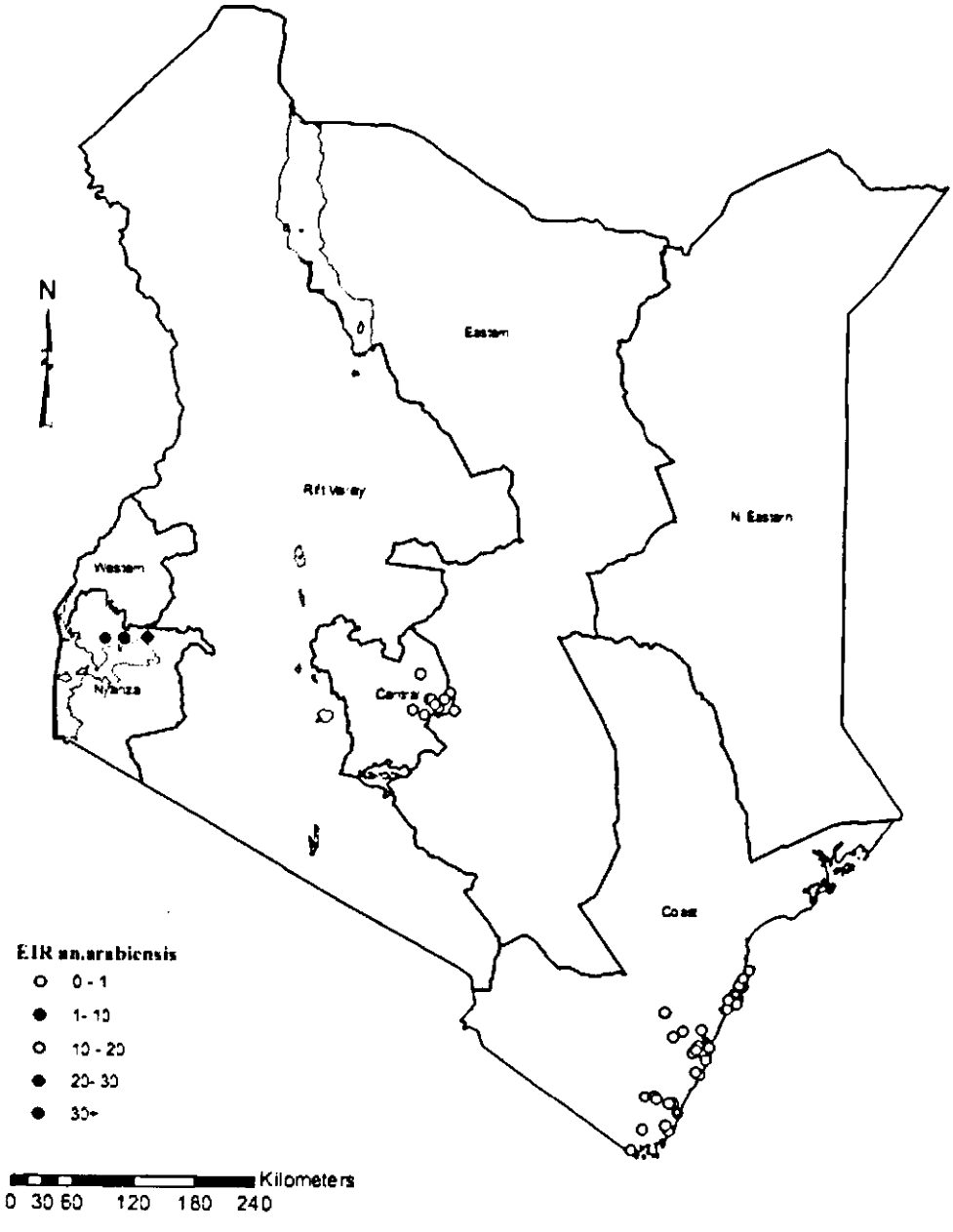


Figure 3.3 Annual Entomological Inoculation Rate for *Anopheles arabiensis* in Kenya

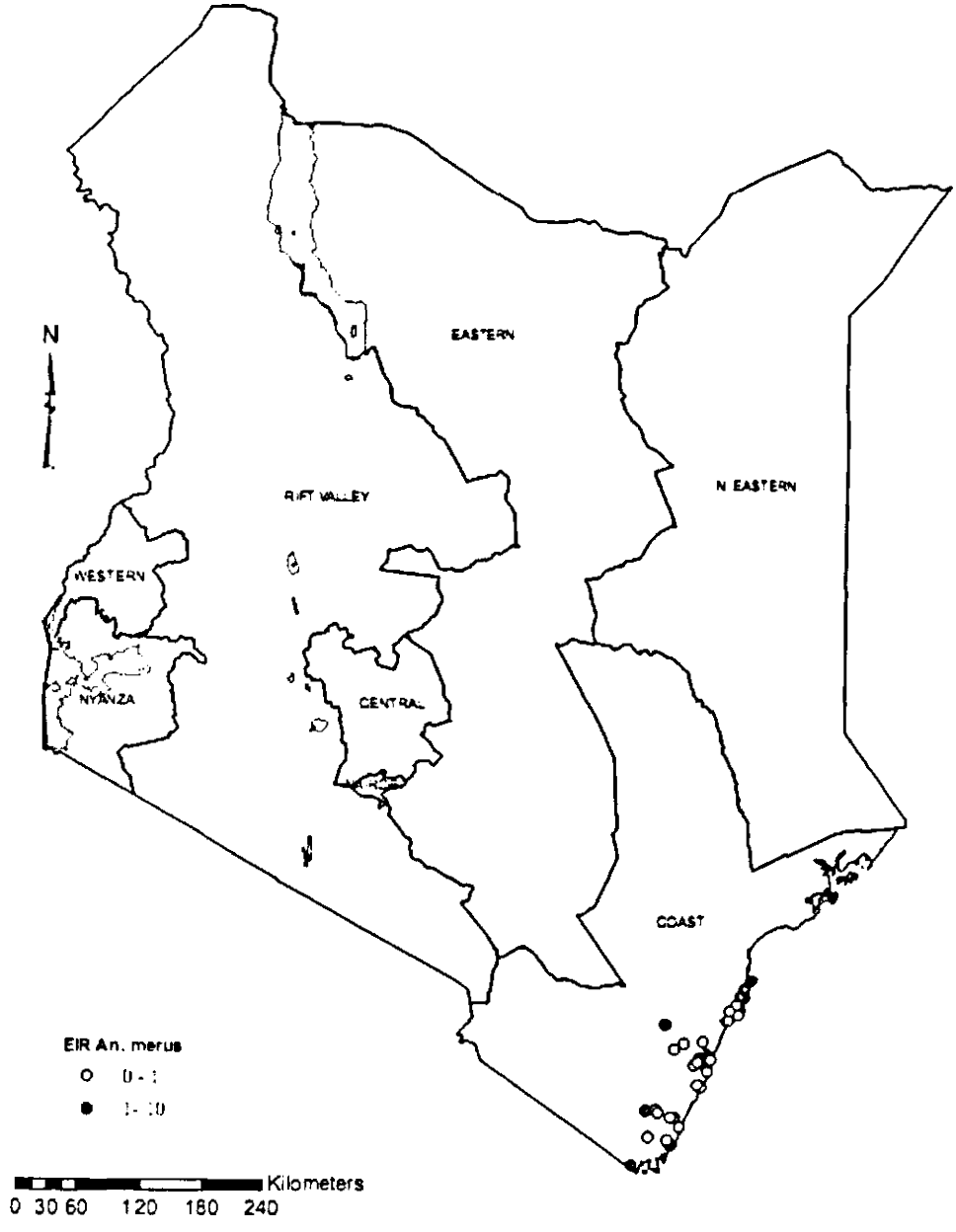


Figure 3.4 Annual Entomological Inoculation Rate for *Anopheles merus* in Kenya

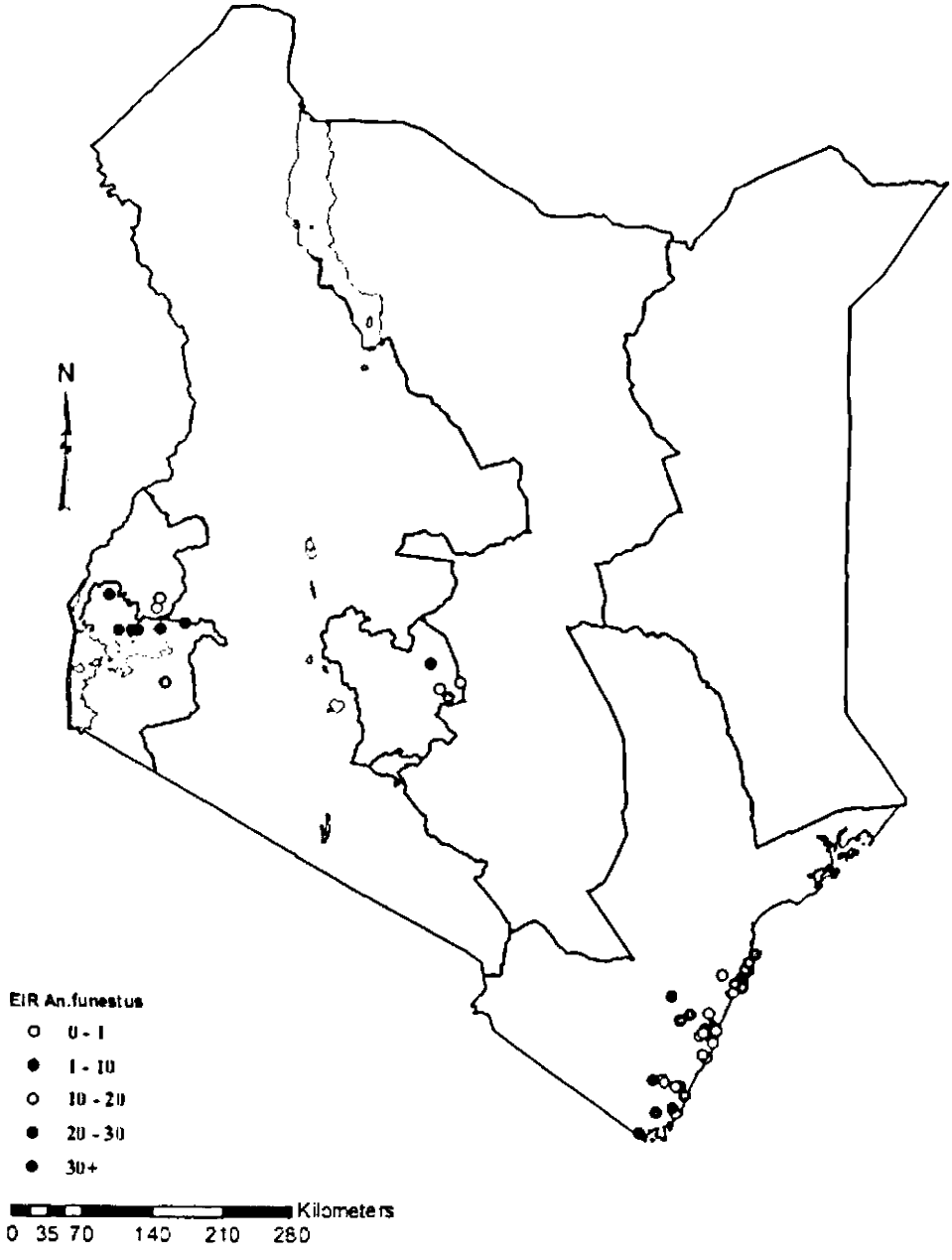


Figure 3.5 Annual Entomological Inoculation Rate for *Anopheles funestus* in Kenya

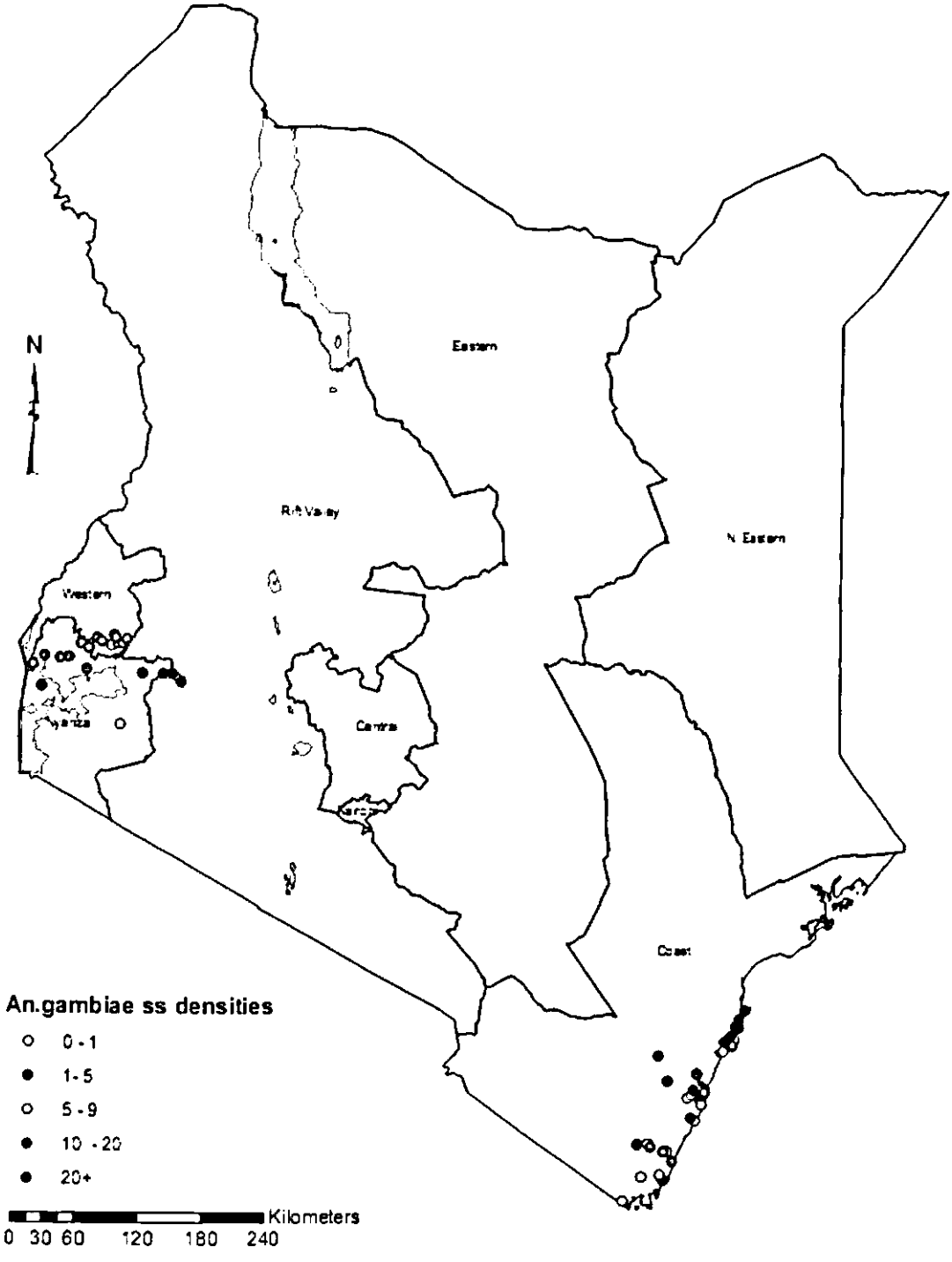


Figure 3.6 *Anopheles gambiae* s.s. densities (Number of mosquitoes/house or trap)

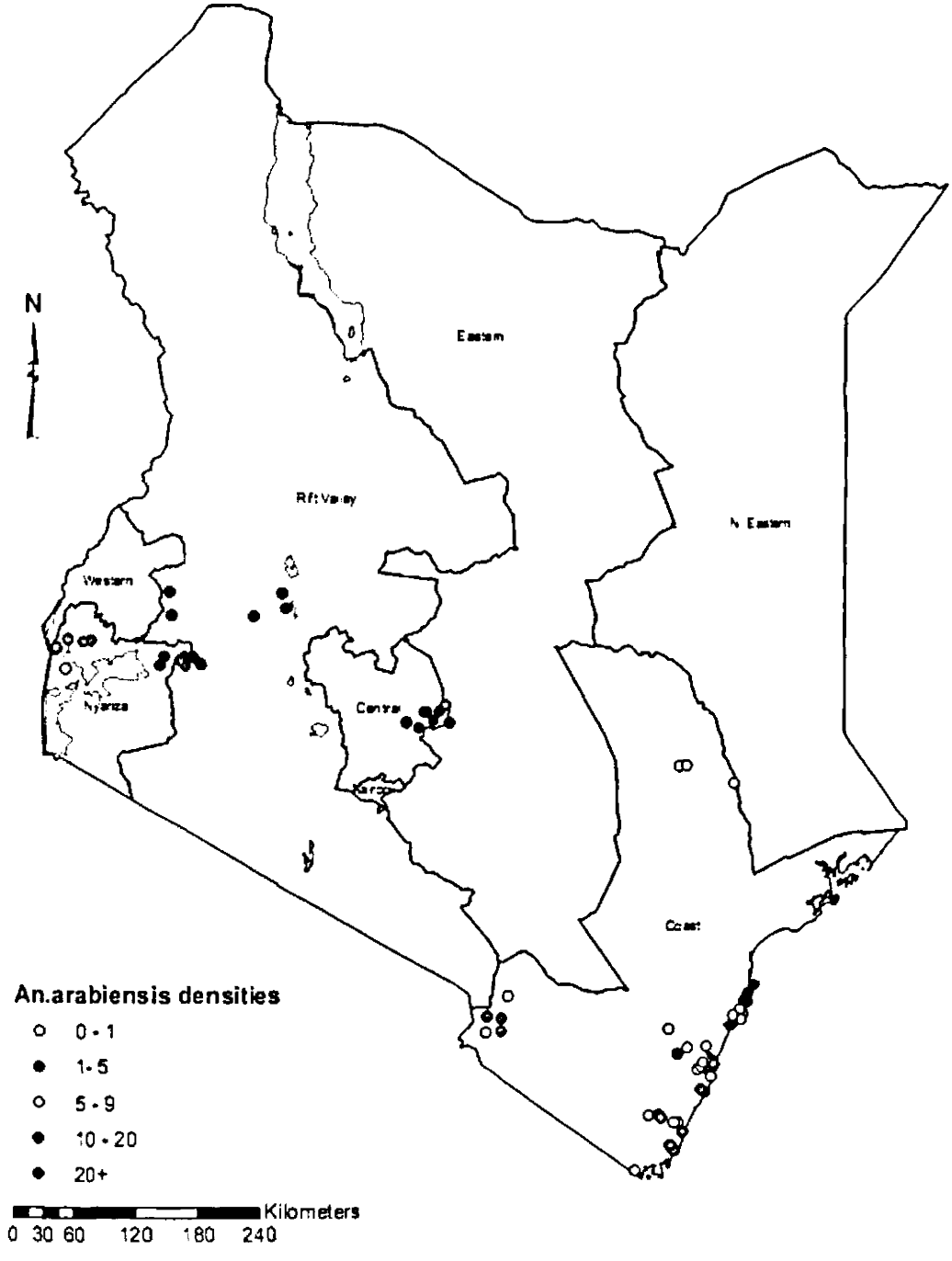


Figure 3.7 *Anopheles arabiensis* densities (Number of mosquitoes/house or trap)

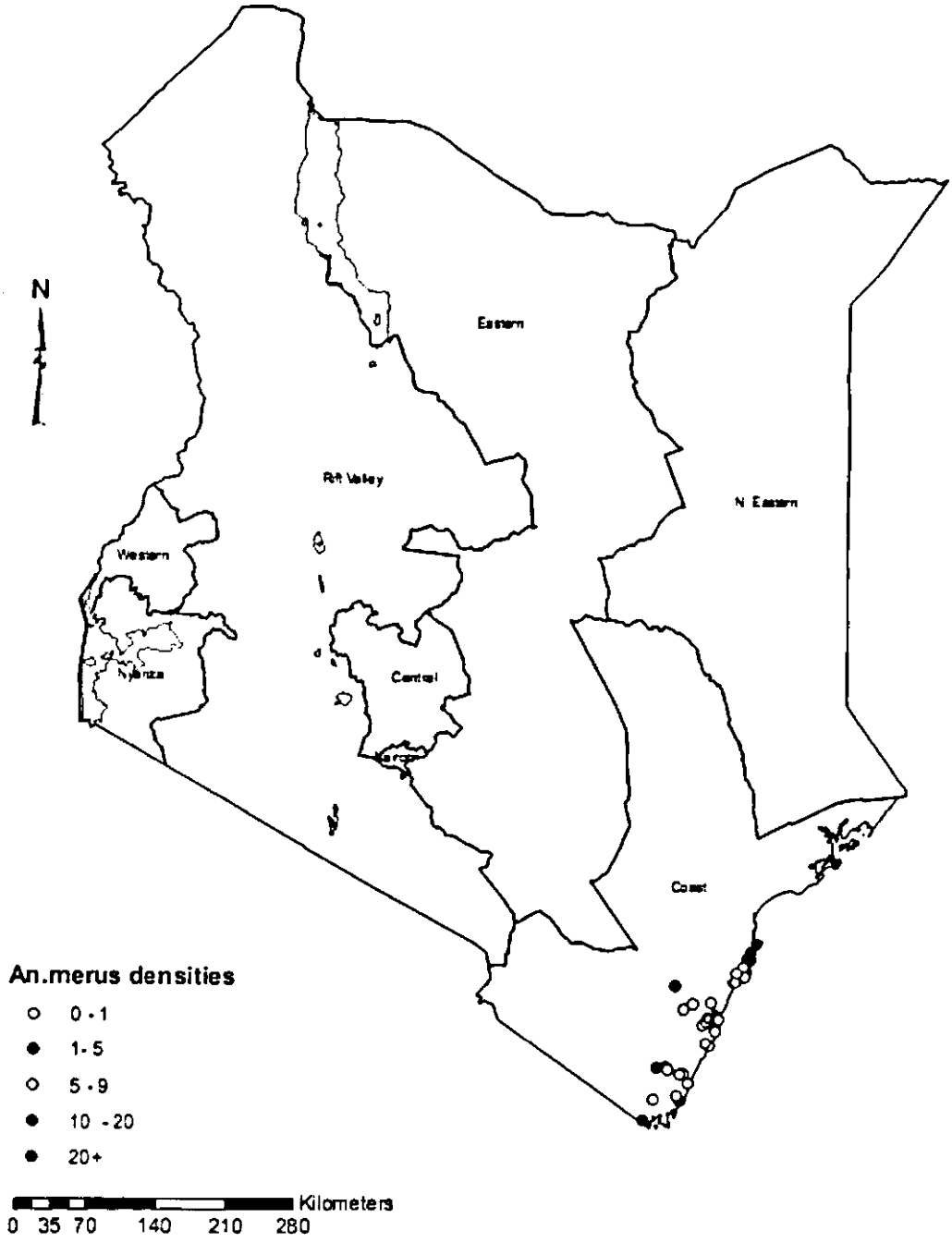


Figure 3.8 *Anopheles merus* densities (Number of mosquitoes/house or trap)

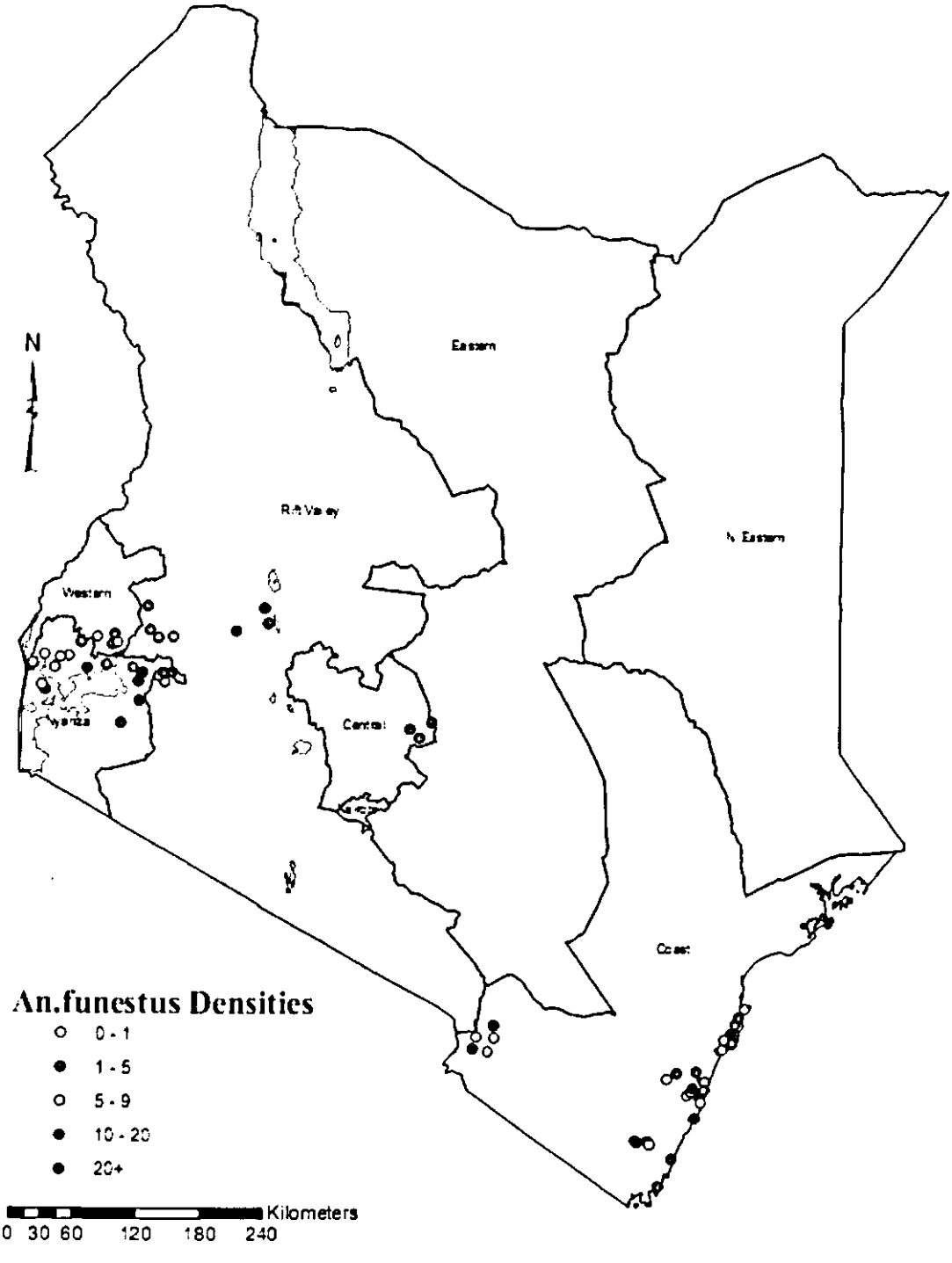


Figure 3.9 *Anopheles funestus* densities (Number of mosquitoes/house or trap)

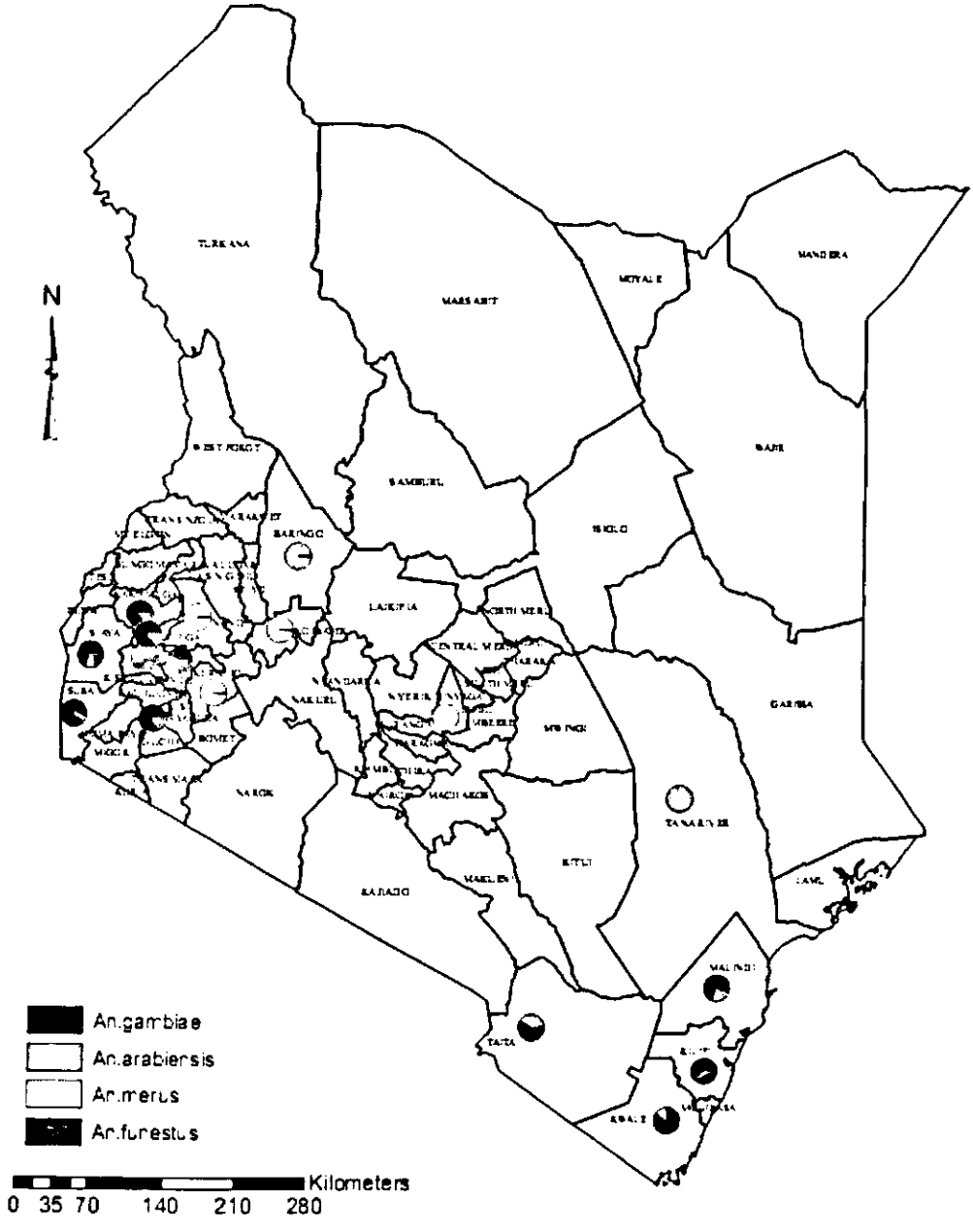


Figure 3.10 Proportions of the main malaria vectors

3.4 Insecticide Resistance Status of the Main Malaria Vector Species

By 2010, a total of four insecticides had been tested for resistance within Kenya around malarious regions of the country with wide range on chemical interventions strategies (Summary of the insecticide resistance status is shown in appendix 4). Resistance to DDT, permethrin, deltamethrin and lambdacyhalothrin had been tested as shown in Figure 3.11, 3.12, 3.13 and 3.14. Only *An. gambiae* s.s. had shown some level of resistance to DDT in Western Kenya but remains susceptible in Coastal region. Other species including *An. arabiensis* and *An. funestus* were susceptible to DDT in all the areas tested including Western and Central Kenya.

Reaction to permethrin had mixed results with *An. funestus* showing susceptibility while *An. arabiensis* and *An. gambiae* s.s. showed susceptibility in some regions of Western Kenya and others it remain resistant with suspected resistance development in other sites e.g. Asembo. However, susceptibility to permethrin among *An. gambiae* s.s. members along the coast region was recorded with some suspicion of resistance to the insecticide. In Western region, *An. gambiae* s.s. was resistant to deltamethrin and in coast province it was susceptible, with suspected resistance development. In *An. arabiensis*, reaction to deltamethrin had mixed results showing susceptibility, resistance and suspected resistance development in Western region. *Anopheles funestus* showed no resistance to lambdacyhalothrin in Western region while *An. gambiae* s.s. was resistant. In Western region, *An. arabiensis* had shown resistance and some areas resistance had been suspected while in Central province it was susceptible against lambdacyhalothrin.

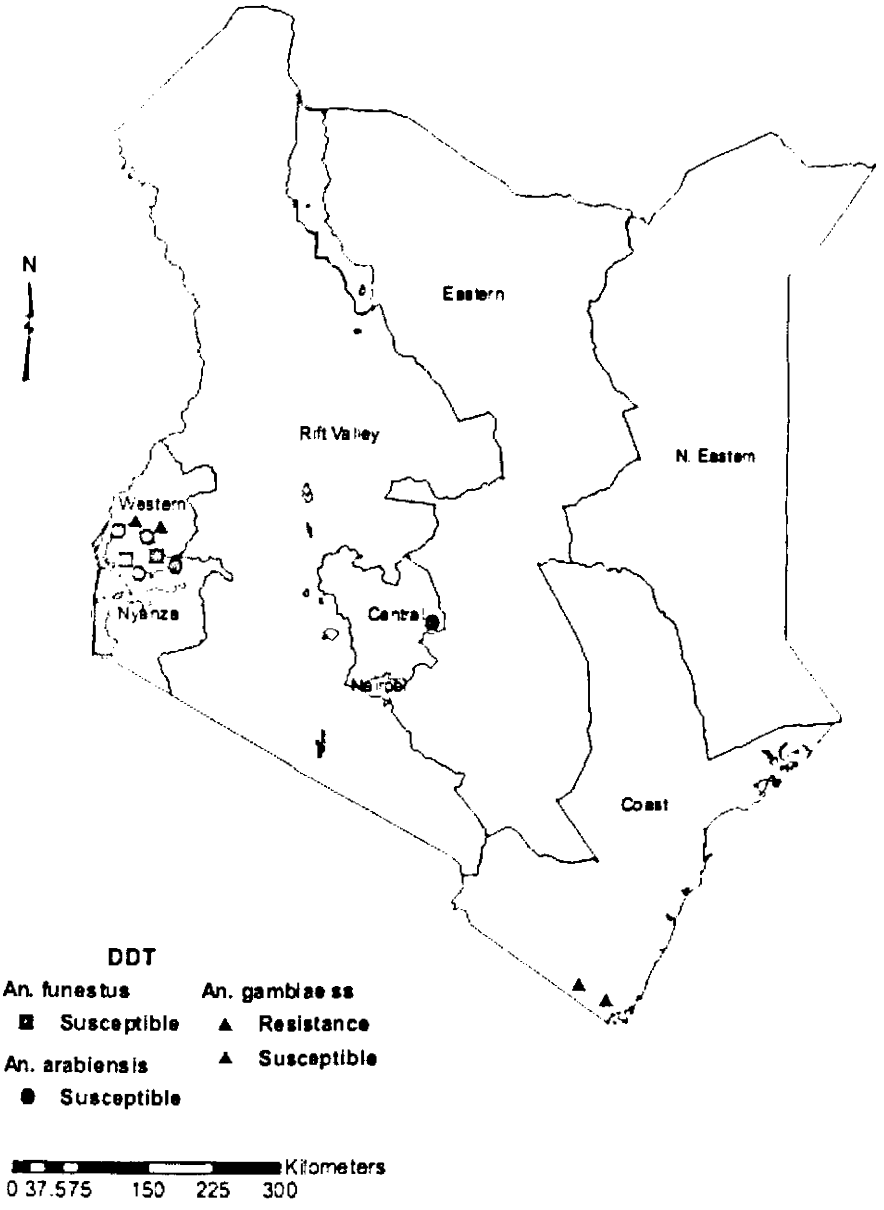


Figure 3.11 Malaria vectors susceptibility status against DDT

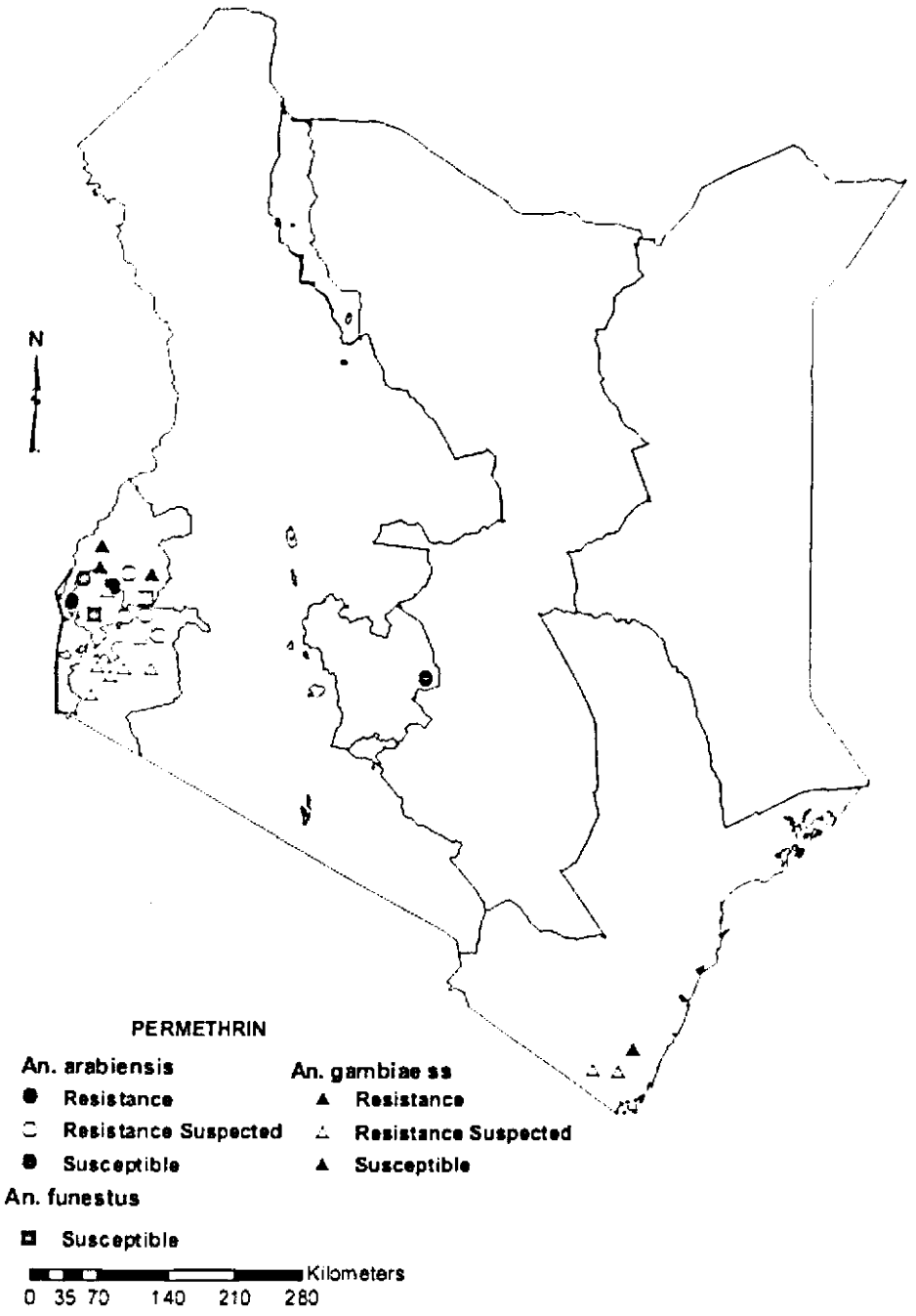


Figure 3.12 Malaria vectors susceptibility status against Permethrin

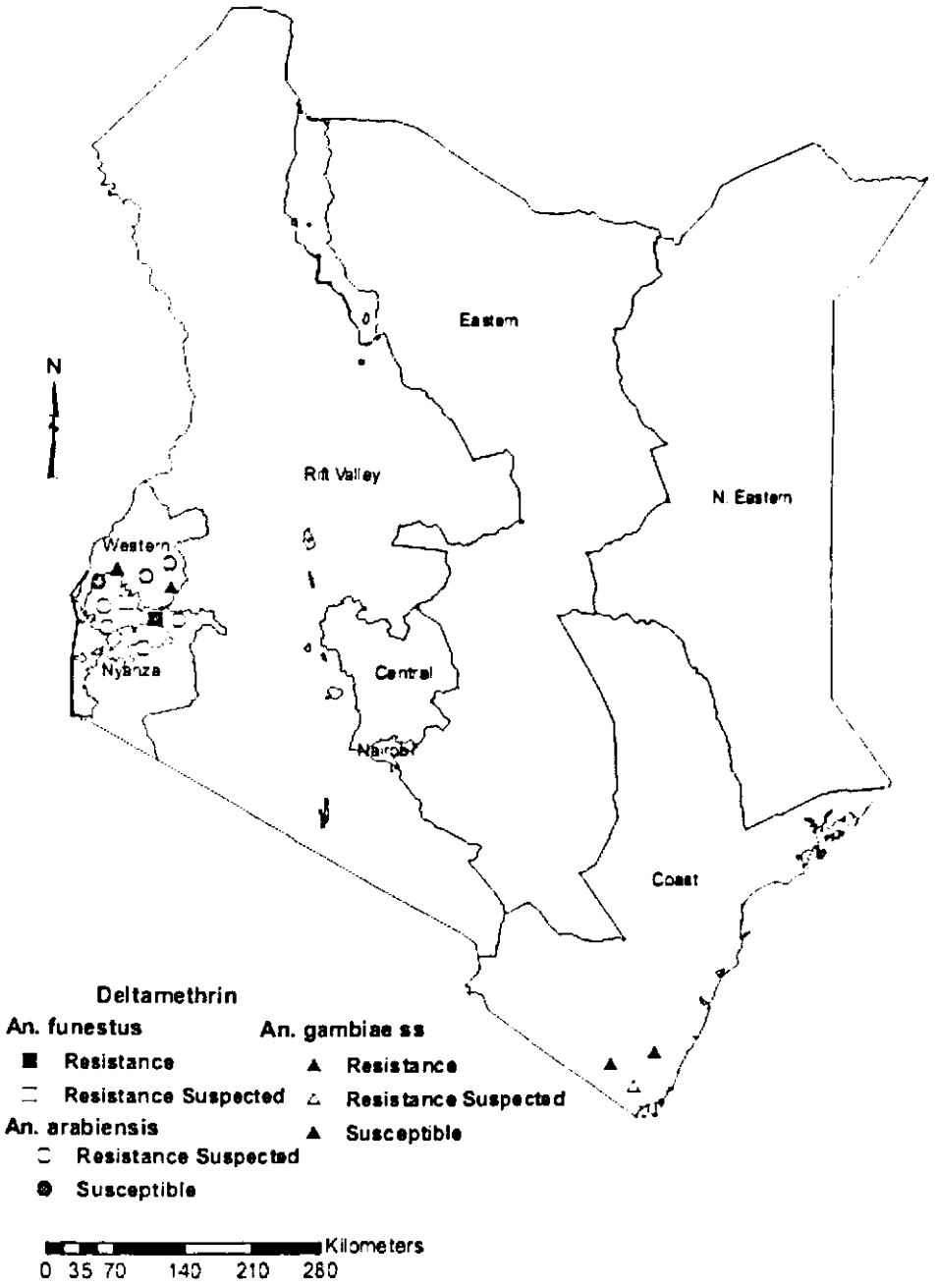


Figure 3.13 Malaria vectors susceptibility status against Deltamethrin

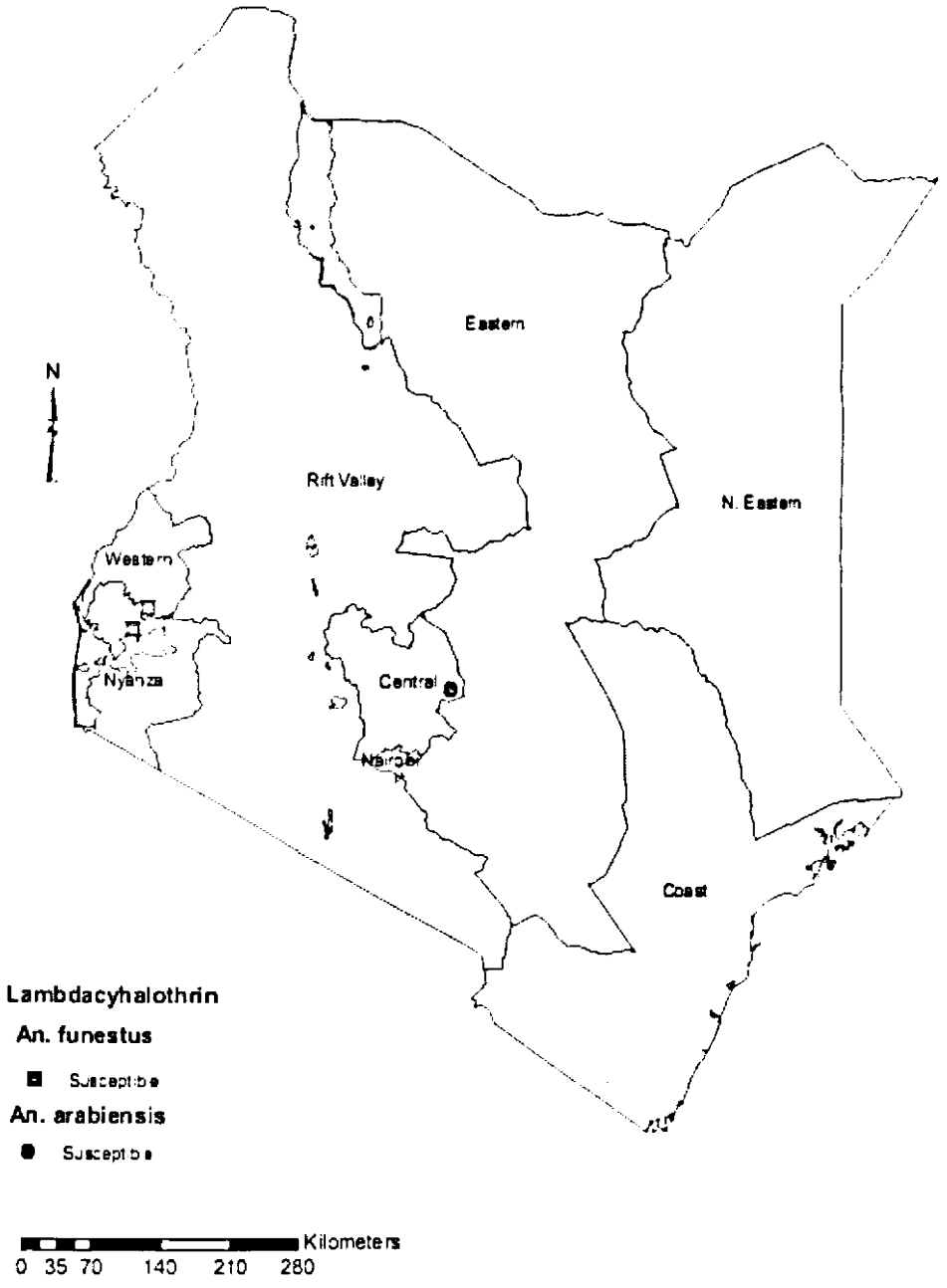


Figure 3.14 Malaria vectors susceptibility status against Lambdacyhalothrin

3.5 Entomological Surveillance

A total of 760 adult, 650 larvae, and 27 pupae mosquitoes were collected in the three study districts along the Kenya coast. All the samples were subjected to morphological identification and sibling species within the *An. gambiae* and *An. funestus* species complexes were further identified through molecular techniques.

3.5.1 Adult Mosquito Diversity

In Taveta district, a total of 357 adult mosquitoes were collected from the 4 villages in Taveta district. Out of these, 148 (41.45%) were morphologically identified as *An. funestus* s.l., 118 (33.05%) *An. gambiae* s.l., 88 (24.64%) *An. coustani* and 3 (0.84%) *An. pharoensis*. Further analysis using Polymerase Chain Reaction (PCR) (Figure 3.15) of the *An. gambiae* s.l. specimens revealed 105 (88.98%) were *An. arabiensis* while 13 (11.01%) could not be determined as shown in Table 3.2. Out of the 148 *An. funestus* s.l. specimen analyzed by PCR 14 (9.46%) were identified as *An. leesoni*, 7 (4.73%) *An. funestus* s.s., 2 (1.35%) *An. parensis* while 125 (84.46%) could not be determined due to amplification failure as shown in Table 3.2. In Taveta, the densities of *Anopheles* were very low ranging from 0 -2.8 mosquitoes/house as shown in Table 3.3. There was a significant site-to-site variation in adult abundance and the Analysis of Variance ($F_{(3)} = 0.14$, $p > 0.05$) further indicates that the 4 villages are different from each other in adult abundance.

In Tana River district, a total of 165 adult mosquitoes were collected from the 5 villages. 101 (61.21%) were *Anopheles* while 64 (38.79%) were *Culex* mosquitoes.

Table 3.2 PCR analysis of the adult *An. gambiae* and *An. funestus* Complexes

District	Site	*An. g* s.l.	*An. g* s.s.	*An. a*	*An. m*	*An. f* s.l.	*An. f* s.s.	*An. lee*	*An. par*
Taveta	Kiwalwa	7	0	55	0	54	6	0	0
Taveta	Mwarusa	2	0	24	0	50	1	0	2
Taveta	Kimorigo	1	0	19	0	10	0	0	0
Taveta	Njoro	3	0	7	0	11	0	14	0
Tana River	V7	0	0	1	0	0	0	0	0
Tana River	V10	0	0	1	0	0	0	0	0
Tana River	Bahati	20	0	76	0	0	0	0	0
Tana River	Chwele	0	0	2	0	0	0	0	0
Kwale	Jego	0	32	74	12	0	0	0	0
Kwale	Kinango	0	23	50	3	0	0	0	0
Kwale	Magaoni	0	7	24	13	0	0	0	0

V4- Village 4 ; V10- Village 10

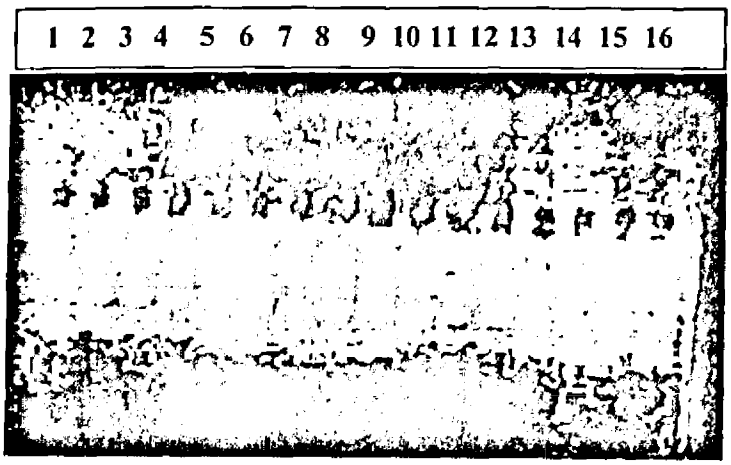


Figure 3.15 The sample DNA in each lane was as follows: 1= didn't amplify ; 2- 15 = *An. arabiensis*; 16 = *An. arabiensis* control.

Table 3.3 Adult mosquito densities in Taveta and Tana River districts

District	Village	*An. gambiae*	*An. funestus*	*An. coustani*	*Culex*
Taveta	Kiwalwa	1.5	0.9	0	*
Taveta	Mwarusa	0.7	0.9	0.05	*
Taveta	Kimorigo	0.4	0	0.05	*
Taveta	Njoro	0.8	2.8	0	*
Tana River	Bahati	2.95	0	0	0.53
Tana River	Chwele	0	0	0	0
Tana River	Village 4	0	0	0	0.33
Tana River	Village 7	*	0	0	*
Tana River	Village 10	*	0	0	*

* Not sampled

Of the *Anopheles* mosquitoes, 100 (99.01%) were *An. gambiae* s.l. and only 1 (0.99%) *An. coustani*. Further analysis using PCR of the *An. gambiae* s.l. specimens revealed 80 (80%) were *An. arabiensis* while 20 (20%) could not be determined due to amplification failure as shown in Table 3.2. In Tana River, the densities of *Anopheles* and Culicines were very low ranging from 0-2.95 mosquitoes/house as shown in Table 3.3.

In Kwale district, a total of 238 adult mosquitoes were collected from the 3 villages consisting of *An. gambiae* s.l. PCR analysis identified 62 (26.1%) as *An. gambiae* s.s., 148 (62.1%) as *An. arabiensis* and 28 (11.8%) as *An. merus* (Table 3.2).

3.5.2 Larval Mosquito Diversity

In Taveta district, a total of 430 *Anopheles* larvae and 7 pupae were collected. A further analysis by PCR identified *An. arabiensis* as the only member of *An. gambiae* complex in Taveta (Table 3.4) and the only 2 specimens for *An. funestus* were non-reactive. The most productive habitats were tyre tracks as shown in Table 3.5. There was a significant site-to-site variation in larvae abundance and the ANOVA ($F_{(4)} = 0.65$, $p > 0.05$) further indicates that the 4 villages are different from each other in larval abundance.

In Tana River district, a total of 137 *Anopheles*, 85 *Culicines* larvae and 20 pupae were collected. A further PCR analysis identified *An. arabiensis* as the only member of *An. gambiae* complex in Tana River while 2 for unexplained reasons were not reactive (Table 3.4). The habitats had low productivity as shown in Table 3.6.

Table 3.4 PCR analysis of larvae *An. gambiae* Complexes

District	Site	Anopheline Larvae Species	
		An. gambiae s.l.	*An. arabiensis*
Taveta	Kiwalwa	9	48
Taveta	Mwarusa	3	93
Taveta	Kimorigo	0	22
Taveta	Njoro	8	10
Tana River	Village 4	0	1
Tana River	Bahati	2	61

Table 3.5 *Anopheles* larvae mosquito densities in Taveta districts

	Kiwalwa	Mwarusa	Kimorigo	Njoro
Stream Pool	0.1	0.6	0	*
Pond	*	1.1	9.4	*
Swamp	*	8.2	1.4	*
Tyre Track	34	*	*	*
Canal	*	*	*	0.7

*Not sampled

Table 3.6 *Anopheles* larvae mosquito densities in Tana River District

	Village 4	Village 10	Chwele	Bahati
Stream Pool	*	*	0	*
Pond	*	*	*	*
Swamp	*	*	*	*
Tyre Track	*	*	*	*
Canal	0.3	0.1	0.3	0.1

*Not sampled

V4- Village 4

V10- Village 10

3.5.3 Host Blood Meal Sources Analysis

In total, 67 blood-fed *Anopheles* mosquitoes that were collected indoors in the 9 sites in Taveta and Tana River districts were all tested for blood meal sources. Majority of the blood-meal sources were of human origin (19.4%), followed by goat (6%) and none tested positive for bovine (Table 3.7). Majority of blood meal sources from Taveta districts had unknown blood meal sources and the possible blood meal sources could have been chicken and donkey whose sources were not tested due to limited resources. The district that had high zoophily was Tana River district (25%) compared to Taveta district (3.6%).

3.5.4 Human Biting Rate, Sporozoite Rate, and EIR

The Human Biting Index ranged between 0.67 and 2 bites per person per night in Taveta and 1.22 in Tana River district. The highest and lowest HBR were both in Taveta district. The *P. falciparum* sporozoite rate in Taveta was 0.9% and 0% in Tana River district. The EIR at the 9 sites ranged from 0 to 0.83 infective bites per person (Table 3.8).

Table 3.7 Blood-meal sources of *Anopheles* mosquitoes in Taveta and Tana River districts

Species	District	Location	# tested	Human(%)	Bovine(%)	Goat(%)	Unknown
An. gambiae	Taveta	Indoors	14	3(21.43)	0(0)	0(0)	11(78.57)
An. funestus	Taveta	Indoors	28	0(0)	0(0)	1(3.57)	27(96.42)
An. coustani	Taveta	Indoors	13	1(7.69)	0(0)	0(0)	12(92.31)
An. gambiae	Tana River	Indoors	12	9(75)	0(0)	3(25)	0(0)

Table 3.8 Summary of the Human-Biting Rate (HBR), *Plasmodium falciparum* sporozoite rate, Human Blood Index (HBI) and Entomologic Inoculation Rate (EIR) collected at 9 sites in Taveta and Tana River districts

District	Village	HBR	Sporozoite Rate	HBI	EIR
Taveta	Kiwalwa	1.02	0	0	0
Taveta	Mwarusa	1.6	1.1	0.07	0.13
Taveta	Kimorigo	0.67	0	0.5	0
Taveta	Njoro	2	2.5	0.17	0.83
Tana River	Bahati	1.22	0	0.75	0

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

The current study has assisted in collating an entomological database showing the distribution and bionomics review of the main malaria vectors in Kenya. From the study, the most widespread *Anopheles* species are *An. arabiensis* and *An. funestus* complex, identified across western, central and coastal regions of Kenya. *An. gambiae* s.s. was largely distributed in western Kenya around Lake Victoria region and along the coastal region with isolated focal presence in central Kenya while *An. merus* was limited to the Kenyan coast. From the geographical distribution maps, it is important to note that malaria vector distribution data was largely limited to areas where intense research has been conducted over time. Those parts of the maps that are blank do not necessarily indicate the absence of the vector, but only that no species identifications have been published from those areas. Worthwhile pointing out from the geographical distribution maps is that some distributional points might be inaccurate because of incorrect map coordinates in the original publications thus placing some data points in the lake e.g. Lake Victoria, Lake Turkana.

In comparison to study conducted by Coetzee *et al.* (2000) on distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex, it was obvious from the maps presented that large areas of the continent had no reliable data on the presence or absence of malaria vectors. Thus, there is an urgent need for baseline surveys to be carried out in these countries and also in Kenya to fill these gaps

In a recent study, vector distribution maps have been updated on continental scale and in addition to showing the presence and absence of malaria vectors, the author used the occurance data combined with expert opinion ranges and a suite of environmental and climatic variables of relevance to anopheline ecology to produce predictive distribution maps (Sinka *et al.*, 2010 a,b). The current study shows only the presence of malaria vectors in Kenya, it is hoped that future surveys will benefit from this current study and produce predictive distribution maps predicting the species range. These data and maps are provided as a dataset to be improved and built upon. Undoubtedly, the process of species distribution mapping will improve, environmental and climatic spatial data will become available at higher resolutions, and more refined understanding of the ecology that limits a given vector distribution attained.

From this study, *An. gambiae* s.s. showed high transmission levels at the coast and western regions while *An. arabiensis* showed high levels of transmission only in Nyanza province. *An. merus* played minor role in malaria transmission at the coast while *An. funestus* showed medium to high levels of transmission at the coast and Nyanza provinces. Transmission levels of malaria vectors are calculated using Entomological Inoculation Rate (EIR). EIR is the number of infectious bites per person per unit time, usually measured or expressed per year. EIR as a direct measure of malaria transmission or sporozoite exposure varies significantly within small geographic areas and should be taken into consideration during malaria control programs (Killeen *et al.*, 2000). The development of sound control strategies for malaria transmission requires a solid understanding of vector dynamics and the factors influencing their spatial and temporal

distribution (Beier *et al.*, 1990). Such information would help to develop early warning systems for predicting malaria epidemics, and for planning control programs based on accurate predictions of their likely effects. Moreover, identification of spatial and temporal variations in vector bionomics and transmission within and among sites, on a district-wide scale, provides useful information for designing effective control programs. From this study, data on transmission level was scanty thus there is need to have a complete and updated data on transmission levels at district level in Kenya.

In western Kenya, high densities of *An. arabiensis* were reported while *An. gambiae* s.s. was generally low in this region. In Nyanza province, high densities of *An. gambiae* s.s. and *An. funestus* were reported. At the Kenyan coast high densities of *An. gambiae* s.s., *An. funestus* and *An. merus* were recorded while *An. arabiensis* reported low densities at the coast. High densities of *An. arabiensis* were reported in Mwea, Kirinyaga district in central Kenya and some drier regions of rift valley. *An. gambiae* s.s. was a dorminant vector in Western region and coastal Kenya mostly in Malindi and Kilifi districts. *An. arabiensis* was dominant in Mwea irrigation scheme in Central Kenya, Tana River district in Bura and Hola irrigation schemes and in Rift Valley province in Kericho district, Nandi district and the drier regions of Baringo and Koibatek districts. The most dorminant vector in Taveta and Kwale districts was *An. funestus*. Climatic factors such as precipitation and temperature are important determinants of the range and relative abundance of individual species of the *An. gambiae* complex (Lindsay *et al.* 1998). *An. gambiae* is usually the predominant species in saturated environments, but *An. arabiensis* is more common in arid areas (Coetzee *et al.* 2000). Distribution of *An. funestus*,

however, is strongly affected by the availability of permanent waters (Evans 1938). Climatic conditions are directly associated with elevation. Temperature decreases as elevation increases, and consequently the abundance and species compositions of malaria vectors may change with elevation.

In Africa, there have been efforts to map the insecticide resistance of the main malaria vectors at nation or continental scale (WHO/ANVR, 2005 Coleman *et al.*, 2006, Santolamazza *et al.*, 2008). These databases are necessary to monitor, detect and manage insecticide resistance.

In Kenya, the first reported case of resistance was in the context of insecticide-treated net use in Western Kenya where reduced knockdown rates were seen (Vulule *et al.*, 1994). Various studies in Kenya have shown *kdr* (Ranson *et al.*, 2000, Stump *et al.*, 2004) and metabolic resistance in some parts of Western region (Chen *et al.*, 2008). Both *kdr* mutation and metabolic resistance confers resistance to pyrethroids which are insecticides used in ITNs and IRS in Kenya.

This study has collated an insecticide resistance database showing the distribution and current insecticide status of the main malaria vectors in Kenya. From the results, insecticide resistance against pyrethroids and DDT is mainly distributed in the western region as shown in figure 21, 22 and 23. The mechanism of resistance as reported by the authors was *kdr* mutation and metabolic resistance (Ranson *et al.*, 2000, Stump *et al.*, 2004, Chen *et al.*, 2008).

Pyrethroids remain effective in the control of malaria vectors in Kenya as shown by the isolated and low *kdr* and metabolic resistances among the vector species. Detection of insecticide resistance should be an essential component of all national malaria control efforts to ensure that the most effective vector control methods are being used.

From the entomological surveillance that was conducted, Taveta district had very low *Anopheles* adult and larval densities. The most productive habitat was the tyre track followed by the pond. There was a significance difference in the adult and larval abundance in the 4 villages. In Tana River district, the *Anopheles* adult and larval densities were also very low. Mosquitoes were sampled during the dry season and this could have contributed to the low densities. The most productive habitat was the canal. In this study, the EIR was between 0 – 0.83 infective bites in Taveta and Tana River districts. In Tana River district, the *Anopheles* mosquitoes had a higher preference for human blood as compared to bovine and goat blood. In Taveta district, the blood source of most *Anopheles* mosquitoes was not determined from the tests that were carried out. This suggests that the *Anopheles* mosquitoes could have fed on other hosts other than the ones that were tested.

CONCLUSION

Distribution database provide valuable data on where the species occur, and can be used by control programme managers in the planning of strategies to combat malaria in their areas. It is obvious from the maps presented here that large areas of the country have no reliable data on the presence or absence of malaria vectors. Therefore, there is an urgent need for baseline surveys to be carried out in these areas on the distribution of the malaria vectors.

From literature, Kenya has scanty data on the insecticide resistance in which more work need to be done to establish the resistance situation.

The entomology studies conducted in Kwale, Taveta and Tana River districts were surveillance studies. A follow up study would be necessary to determine the mosquito abundance, transmission level and the insecticide resistance in the 3 districts. This will help fill the gaps that have been identified from the maps that have been generated.

5.0 RECOMMENDATIONS

The national malaria surveillance programme should adopt a standardized technique for speciation.

Detection of insecticide resistance should be an essential component of all national malaria control efforts to ensure that the most effective vector control methods are being used.

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7.0 APPENDICES

Appendix 1

FIELD DATA SHEET-ADULT MOSQUITO COLLECTION

Collection site _____ **Collection Date** _____ **Collection Technique** _____

House		Co-ordinates		*An. G* F				*An. G* M	*An. F* F				*An. F* M	Other *Anopheles* F				Other *Anopheles* M	*Culicines*	Remarks
#	# Sleepers	Long-E	Lat-S	UF	BF	H G	G	#	UF	B F	H G	G	#	UF	BF	H G	B	#		

Key: UF=Unfed, BF=Blood Fed, HG=Half Gravid, G=Gravid, F=Female, M=Male

Appendix 2

FIELD DATA SHEET-LARVAE MOSQUITO COLLECTION

Collection site _____ Date Collection _____

Habitat Type	Habitat Size (m)			# Dips	Long-E	Lat-S	*Anopheles*				*Culicines*				Pupae	Remarks
	L	W	D				L1	L2	L3	L4	L1	L2	L3	L4	#	

L=Length, W= Width, D=Depth

Appendix 3

MALARIA VECTOR BIONOMICS – KENYA

		An. gambiae s.s.	*An. arabiensis*	*An. merus*	*An. funestus*
GEOGRAPHICAL DISTRIBUTION					
WHO regional office	AFRO	7	7	7	7
Regionally dominant vector in Macdonald (1957) malaria zone (1: North American, 2: Central American, 3: South American, 4: North Eurasian, 5: Mediterranean, 6: Afro-Arabian (desert),7: Afrotropical (formerly Ethiopian), 8: Indo-Iranian, 9: Indo-Chinese hills, 10: Malaysian, 11: Chinese, 12: Australasian.					
LARVAL SITE CHARACTERISTICS					

Light intensity	Heliophilic	Yes	Yes	Yes	No
	Heliophobic	No	No	No	Yes
Salinity	High (Brackish)	No	No	Yes	No
	Low (Fresh)	Yes	Yes	No	Yes
Turbidity	Clear	Yes	Yes	Yes	Yes
	Polluted	Yes	Yes	Yes	No
Movement	Still or Stagnant	Yes	Yes	Yes	Yes
	Flowing	No	No	No	No
Vegetation	Higher plants, algae etc	Yes	Yes	Yes	Yes
	No vegetation	No	No	No	No
NATURAL AND MAN MADE LARVAL SITES					
Large natural water collections	Lagoons	No	No	Yes	No
	Lakes	No	No	No	Yes
	Marshes	No	No	Yes	No

	Bogs	No	No	Yes	No
	Slow flowing rivers	No	No	No	No
Large man-made water collections	Borrow pits	Yes	Yes	No	Yes
	Rice fields	Yes	Yes	No	Yes
	Fish ponds	Yes	Yes	No	Yes
	Irrigation channels	Yes	Yes	No	Yes
Small natural water collections	Small streams	Yes	Yes	No	No
	Seepage springs	Yes	Yes	No	No
	Pools	Yes	Yes	No	Yes
	Wells	Yes	Yes	No	No
	Depressions in the ground	Yes	Yes	No	No
Small man-made water collections	Overflow water	Yes	Yes	No	No
	Irrigation ditches	Yes	Yes	No	No
	Borrow pits	Yes	Yes	No	No
	Wheel ruts	Yes	Yes	No	No

	Hoof prints	Yes	Yes	No	No
	Puddles near rice fields	Yes	Yes	No	No
Artificial sites	Empty cans, shells etc.	Yes	Yes	No	No
ACTIVITY					
Feeding habit	Anthropophilic	Yes	Yes	Yes	Yes
	Zoophilic	No	Yes	No	No
Biting habit	Exophagic	No	Yes	No	Yes
	Endophagic	Yes	Yes	Yes	Yes
Biting time	Day	No	No	No	No
	Dusk				
	Night				
	Dawn				
Pre-feeding resting habit	Exophilic	No	Yes	No	
	Endophilic	Yes	Yes	Yes	
Post-feeding resting habit	Exophilic	No	Yes	Yes	No

	Endophilic	Yes	Yes	No	Yes
Flight range (km)	Daily				
	Lifetime				

Adapted from Takken & Knols (1990)

Appendix 4

INSECTICIDE RESISTANCE STATUS OF THE MAIN MALARIA VECTORS IN KENYA

District	Species	Insecticide	Insecticide Status (Site)
Kisumu	*An. gambiae*	Permethrin	Resistance Suspected (6)
Bondo	*An. gambiae*	Permethrin	Susceptible (1)
Busia	*An. gambiae*	Permethrin	Resistance (1)
Kakamega	*An. gambiae*	Permethrin	Resistance (1)
Kisumu	*An. gambiae*	Permethrin	Resistance (1)
Kwale	*An. gambiae*	Permethrin	Resistance Suspected (2)
Kwale	*An. gambiae*	Permethrin	Susceptible (1)
Kirinyaga	*An. arabiensis*	Permethrin	Susceptible (1)
Bondo	*An. arabiensis*	Permethrin	Susceptible (1)
Bondo	*An. arabiensis*	Permethrin	Resistance Suspected (2)
Kakamega	*An. arabiensis*	Permethrin	Resistance (1)
Kisumu	*An. arabiensis*	Permethrin	Resistance Suspected (1)
Kisumu	*An. funestus*	Permethrin	Susceptible (1)
Nyando	*An. funestus*	Permethrin	Susceptible (1)
Bondo	*An. funestus*	Permethrin	Susceptible (1)
Bungoma	*An. gambiae*	Deltamethrin	Resistance (1)
Busia	*An. gambiae*	Deltamethrin	Resistance (1)
Kakamega	*An. gambiae*	Deltamethrin	Resistance (1)

Kisumu	*An. gambiae*	Deltamethrin	Resistance (1)
Kwale	*An. gambiae*	Deltamethrin	Resistance Suspected (1)
Kwale	*An. gambiae*	Deltamethrin	Susceptible (2)
Bondo	*An. arabiensis*	Deltamethrin	Resistance Suspected (1)
Bungoma	*An. arabiensis*	Deltamethrin	Resistance (1)
Busia	*An. arabiensis*	Deltamethrin	Susceptible (1)
Kakamega	*An. arabiensis*	Deltamethrin	Susceptible (1)
Kisumu	*An. arabiensis*	Deltamethrin	Resistance Suspected (1)
Bungoma	*An. gambiae*	Lambdacyhalothrin	Resistance (1)
Busia	*An. gambiae*	Lambdacyhalothrin	Resistance (1)
Kakamega	*An. gambiae*	Lambdacyhalothrin	Resistance (1)
Kisumu	*An. gambiae*	Lambdacyhalothrin	Resistance (1)
Bungoma	*An. arabiensis*	Lambdacyhalothrin	Resistance (1)
Busia	*An. arabiensis*	Lambdacyhalothrin	Resistance Suspected (1)
Kakamega	*An. arabiensis*	Lambdacyhalothrin	Resistance (1)
Kisumu	*An. arabiensis*	Lambdacyhalothrin	Resistance Suspected (1)
Bungoma	*An. gambiae*	DDT	Resistance (1)
Kakamega	*An. gambiae*	DDT	Resistance (1)
Kisumu	*An. gambiae*	DDT	Resistance (1)
Kwale	*An. gambiae*	DDT	Susceptible (2)
Kakamega	*An. arabiensis*	DDT	Susceptible (1)
Kisumu	*An. arabiensis*	DDT	Susceptible (1)